THE BLOOD IN HEALTH AND DISEASE

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R. J. M. BUCHANAN

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THE BLOOD IN HEALTH AND DISEASE

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Dedicated
TO
MY WIFE

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PREFACE

THIS book has been produced in the hope that it may prove useful to medical students and practitioners who may be desirous of including hæmatology in their clinical studies and practice. The plates have been drawn by myself from blood specimens prepared by me, with a view to providing a faithful atlas of the principal morphological changes which are to be found in abnormal blood states. I appreciate the care which the publishers have expended over them in order to obtain correct colour pictures. The cytological work has been done in the Department of Cytology of the Liverpool University and Royal Infirmary. I have to express my thanks to Sir James Barr for placing clinical material at my disposal; to Dr. Hurter for specimens of leukanæmia; to Dr. Addis for kindly allowing me the use of his manuscript on coagulation; to Dr. Blair Bell for a personal description of his method of calcium estimation; and to my wife for her assistance in drawing up the index.

No attempt has been made to include malarial and similar parasitic diseases, as they have now passed into the special domain of tropical diseases. To provide a complete bibliography on the blood in health and disease would be quite beyond the scope of this work. I have obtained much valuable assistance from the works of Ewing, Da Costa, Cabot, Buckmaster, Ehrlich, Lazarus, v. Noorden, Pincus and others, which I have freely consulted, as well as various text-books on Medicine and original articles. For assistance in obtaining blocks for the illustration of the instruments I am indebted to Mr. T. Hawksley of London and Messrs Alexander and Fowler of Liverpool.

R. J. M. BUCHANAN.

LIVERPOOL, 1909.

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CHAPTER I

General Characters of Normal Blood—Examination of Fresh Blood.

NORMAL BLOOD

The blood is a tissue consisting of two principal elements—the plasma or liquor sanguinis and the corpuscles. To the naked eye it has a red colour, varying from the scarlet of arterial to the dark purple of venous blood. In thin layers it appears yellowish-red by transmitted light, but if dried in a film on glass it appears iridescent. The red colour is due to the hæmoglobin of the red corpuscles, and varies with their relative number and the amount of hæmoglobin which they contain. The corpuscles render the blood opaque, and the opacity varies with their relative number and richness in hæmoglobin. Should the colouring matter be dissolved out of the corpuscles into the plasma, the blood becomes less opaque and "laky" in appearance. The plasma is a yellowish-looking fluid consisting largely of proteid material in the form of fibrinogen, serum albumin and serum globulin. Its solids amount to about 10 per cent., three-fourths of which are proteids. It is alkaline or acid in reaction, according to the indicator used, and has a specific gravity ranging from 1026 to 1030.

The corpuscles comprise about two-fifths of the volume of the blood; they are suspended in the plasma, and move with it in the circulation. They are cellular elements of two distinct varieties; the red corpuscles or erythrocytes, and the white corpuscles or leucocytes. Besides these, and classified with them as corpuscular elements, are the blood platelets and blood dust or hæmoconia. As yet it is unsettled whether these are true cell elements. The mineral matter of the blood is present in the form of salts, comprising chlorides of sodium and potassium, sodium carbonate, phosphates of sodium, lime and magnesium. Sodium chloride is the chief of these, and amounts to between 60 and 90 per cent. of the mineral matter. The gases, oxygen, nitrogen and carbon dioxide are present in the blood; oxygen in combination with the hæmoglobin, nitrogen in simple solution, and the carbon dioxide in combination with sodium as carbonate. One hundred volumes of blood contain approximately sixty volumes of gases. In arterial blood oxygen is present to the extent of twenty, and carbon dioxide to forty volumes; in venous blood there are ten of oxygen to fifty of carbon dioxide; while the nitrogen, amounting to one or two volumes, is the same for both.

At a variable period subsequent to its withdrawal from the body, certain changes take place in the blood which result in its coagulation. A reticulum consisting of fibrin forms in it, which entangles in its meshes the corpuscular elements. These together constitute the clot which contracts and separates out from the fluid material, which is now called the serum. This is a straw-coloured fluid with a specific gravity of about 1026 and an alkaline reaction. It contains practically the same proteids and solids as the plasma, but instead of fibrinogen, which was present in the latter, a new substance is substituted, the fibrin ferment.

Blood has a peculiar and characteristic odour which soon disappears after withdrawal from the vessels. The somewhat oleaginous feel to the touch which blood gives is soon lost, as its coagulation renders it viscid and sticky. The specific gravity of the blood as a whole averages about 1060, and its reaction is identical with that of the serum or plasma, from the presence of sodium carbonate and disodic phosphate.

The blood carries in it several extractives—urea, uric acid, creatin, creatinin, xanthin, hypoxanthin, cholesterin, sugars, fats, soaps and enzymes. Besides these are the innumerable bodies which are more or less constantly being formed for the protection of the organism against injurious influences from outside or even from itself. Every tissue of the body is continually giving something to the blood, so that it would seem that there could be no limit to its constituents or their fluctuations. It is a difficult matter to estimate the whole amount of blood in the body, but it has been calculated at about one-thirteenth of the body weight. This estimate, according to more recent methods, appears to be too high, and that the total mass of blood is about one-twentieth of the body weight.

EXAMINATION OF FRESH BLOOD

Method of obtaining the specimen.—The blood is generally obtained by puncturing the skin in a convenient locality, the finger tip, the lobe of the ear, or the dorsum of the terminal phalanx of the middle finger just above the nail. I prefer the latter as the blood flows easily, and its rate can be conveniently hastened or increased by simply flexing the terminal phalanx on the second. After cleansing the finger with soap and water, followed by alcohol and ether, a puncture is made by means of a sterilized needle or special stilette for the purpose. Any good sized surgical needle will do ; or, what is preferable, a glass needle made by fusing a capillary glass tube in the spirit flame and drawing it apart. Broken off short this provides a sharp, graduated and sterile point, which produces a puncture from which the blood flows freely. Should a relatively large amount of blood be required, it may be syphoned off into a Wright's curved tube, or drawn into a sterile pipette and then sealed. Venesection, wet cupping, or aspiration of a vein with a sterile syringe may be resorted to if still larger quantities be required for special purposes. Wright's capsules are made of short lengths of glass tubing curved round at one end and drawn out to capillary dimensions at both. To fill one the point at the curved end is applied to the drop of blood while held in a horizontal position. The blood flows round the curve into the body of the tube. When sufficient has been collected, the straight end is warmed and then sealed ; as the tube cools, the blood in the curved end passes over into the body of the tube, when the inlet can also be sealed.

For immediate examination with the microscope the drop of blood is touched with a clean microscope slide and covered with a No. 1 cover-slip. To prevent rapid drying, a rim of melted paraffin or vaseline should be smeared round the margin. Thick gum arabic does very well. The specimen so obtained can be examined at once under the microscope, using a $\frac{1}{12}$th inch oil immersion lens and good ocular. Attention should be paid to the proper illumination of the specimen by the substage condenser, by focussing it until the corpuscles are clearly defined. The results to be obtained from one's examination of the fresh specimen are limited, but certain features of normal and abnormal conditions may at once be recognized. By the examination of a series of specimens of normal blood, acquaintance is made of the general morphological characters, colour and distribution of the erythrocytes and the relative number and characters of the leucocytes, the appearance of the blood platelets, the present of hæmoconia, and the formation of fibrin. The erythrocytes will be recognized as biconcave yellow-coloured discs, often clinging together in the peculiar formation of rouleaux; the leucocytes as silvery-looking bodies larger than the erythrocytes, and of varied size; some small, others larger, of which the majority are finely granular and with an irregularly shaped nucleus. A few are more granular and highly refractile, but the nucleus is seen with difficulty. In some of the leucocytes amœboid movement can be recognized. The hæmoconia or blood dust occurs in the form of minute highly refractile granules showing active Brownian movement. After a period of time fine fibrils can be seen radiating from grape-like clusters of minute, non-refractile bodies; the former constitute the fibrin deposit and the latter the blood platelets.

From a practical knowledge of the normal appearances of blood, within certain limits, departures therefrom can be easily recognized. In the flowing drop, changes of colour, under the microscope, abnormal variations in the shape and colour of the erythrocytes may be seen associated with pathological conditions. A limited opinion may be formed of any decrease in their number or any lack of rouleaux formation or fibrin deposition. With reference to the leucocytes, practice will assist in judging of their numerical gain or loss. One must remember that any erythrocytic loss may give a false impression of leucocytic gain. It is not difficult to determine a marked increase in the number of leucocytes, or even to identify the majority of them. Although the expert may be able to differentiate the various forms of leucocytes with fair accuracy, it is advisable to proceed with a fuller examination of stained specimens in order to eliminate any possible chance of error. Degenerative changes are difficult to determine in the leucocytes in fresh specimens, but vacuolation, breaking up and seething of the granules may be detected. One should note the rapidity and amount of fibrin deposit, any lack or excess of blood platelets, and the presence, if any, of pigment in cells or plasma. The presence of parasites, fat and crystals can be easily determined and studied.

CHAPTER II

THE COLOURING MATTER OF THE BLOOD AND ITS VARIATIONS—ESTIMATION OF HÆMOGLOBIN—METHODS AND INSTRUMENTS USED—HÆMOGLOBINOMETERS.

THE COLOURING MATTER OF THE BLOOD AND ITS VARIATIONS

THE normal colour of the blood, as previously described, is due to the presence in the erythrocytes of a proteid material, the **hæmoglobin**, which contains about 4 per cent. of a ferruginous pigment, **hæmochromogen.** It is combined with the stroma of the erythrocytes, and, constitutes about nine-tenths of their bulk. In the adult, hæmoglobin comprises about 14 per cent. of the whole blood. With oxygen it combines in an unstable manner to form oxyhæmoglobin, and in this condition is present in both arterial and venous blood, but in the latter less in amount. Passing through the capillaries as oxyhæmoglobin, the looseness of its combination with oxygen permits of easy dissociation, and the oxygen is given up to the tissues. Further, it is easily acted upon by deoxidizing agents and changed to reduced hæmoglobin. In this form it is largely present in venous blood, and gives to it its deeper and more purple colour in comparison with the brighter and redder arterial hue.

Under certain conditions hæmoglobin will crystallize out in red or reddish-green rectangular plates. When separated from the erythrocytes, these crystals give the spectrum of reduced hæmoglobin, in contradistinction to those derived from the blood of the lower animals, which give the spectrum of oxyhæmoglobin. Oxygen may combine with hæmoglobin more firmly, although in the same proportions as in oxyhæmoglobin, to form the substance known as methæmoglobin. It gives to the blood a dull brown or chocolate colour.

By the action of strong alkalies or acids hæmoglobin is decomposed into two bodies—**hæmatin,** which contains iron, and an albuminous material of the nature of a globulin. Acted upon by hydrochloric acid, hæmatin combines with it to form a hydrochloride, and crystallizes in rhomboidal prisms or whetstones, of a yellowish-red or brownish-black colour with a metallic lustre. They are called **hæmin** or Teichmann's crystals. Many of the crystals exhibit a lipped projection on one side. They often occur in clusters. They produce effervescence with peroxide of hydrogen. They are insoluble in water, alcohol, ether, chloroform, and dilute acids. An easy method of obtaining them from any solution containing hæmoglobin is, after concentration, to add to a drop of the solution on a microscope slide a small crystal of common salt and a drop or two of glacial acetic acid; on warming under a cover-glass and allowing to cool, the crystals form and can be easily detected with the aid of a microscope.

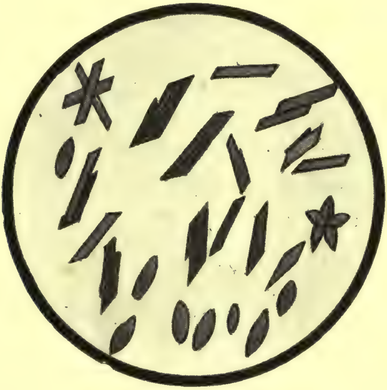

FIG. 1.—HÆMIN CRYSTALS.

The iron can be removed from hæmatin by the action of strong sulphuric acid, and a substance obtained which is akin to urobilin. It is known as **hæmatoporphyrin,** and is found occasionally as an abnormal constituent of urine. The prolonged use of sulphonal, trional and tetronal is said to lead to its presence in the urine.

Another iron-free derivative of hæmatin is **hæmatoidin,** which separates out from old blood extravasations in the form of rhombohedral crystals. It is considered identical with urobilin.

Any lack of hæmoglobin, and, as has been described above, certain chemical combinations into which it may enter, produce deviations from the normal blood colour, and may be seen when blood is drawn. In anæmias the blood is pale and watery-looking ; in leukæmias it may have a creamy appearance. The colour approaches that of venous blood in conditions associated with dyspnœa and consequent deficiency of oxygenation. The presence of fat gives a pinkish-creamy appearance to the blood in diabetes mellitus ; on standing the fat separates out in extreme cases as a cheesy-looking layer. In jaundice the blood may be tinged yellowish-red.

In anæmias the loss of colouring matter or **oligochromæmia** may be due to a simple diminution of hæmoglobin, the number of erythrocytes remaining about normal, or to a loss due to reduction in the number of the erythrocytes.

Oligochromæmia is associated with all anæmias from any cause. Without absolute loss a relative diminution may be produced by dilution of the plasma ; on the other hand, a relative increase may arise from plasma concentration. Under certain pathological conditions the hæmoglobin may leave the erythrocytes and become dissolved in the plasma—**hæmoglobinæmia**. This occurs when the erythrocytes are placed in an excess of hypotonic saline solution ; the hæmoglobin passes out, but the stroma of the erythrocytes is not dissolved. All the erythrocytes do not act alike in parting with their hæmoglobin in hypotonic solutions. Hæmoglobinæmia may be accompanied by the excretion of the hæmoglobin in the urine, as in paroxysmal hæmoglobinuria and epidemic hæmoglobinuria of the newly born. It seems, however, that in the former condition the resistance of the erythrocytes to saline solutions is normal.[1]

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[1] Chvostek *Uber das Wesen der paroxysmalen Hæmoglobinurie*, Wien, F. Deuticke, 1894.

The solution of hæmoglobin in the plasma, or "laking" of the blood, can be easily produced by the action of excessive heat or cold. If blood be drawn into a glass tube and heated, not sufficiently to coagulate the plasma, then sealed, allowed to clot, and centrifugalized, the serum, instead of being a pale straw colour, will be coloured red by the hæmoglobin. The same result will ensue if, instead of heating. the tube be placed in a refrigerator for twenty-four hours,

By transfusing the blood of one animal into another of a different species hæmoglobinæmia is produced, also by poisons which act upon the blood, such as arseniuretted hydrogen, sulphuretted hydrogen, potassium chlorate, carbolic, hydrochloric, sulphuric and pyrogallic acids, and the nitro-benzines. Certain synthetical drugs like antipyrin, phenacetin, acetanilid, act in a similar way, as do snake venom, some vegetable glucosides and certain poisonous fungi.

Exposure of the body to extreme heat or cold may produce this condition and it is not uncommonly associated with the infectious fevers, scurvy and Raynaud's disease.

The condition of methæmoglobinæmia is also produced by the action of certain drugs, chief among which are anilin, potassium chlorate, the nitrites and nitrite of amyl.

Carbon monoxide gas combines with hæmoglobin, and is supposed to form a fixed compound which paralyses the oxygen-carrying power of the erythrocytes. This combination produces a characteristic cherry-red colour of the blood. The condition is commonly associated with gas poisoning, and produces characteristic post mortem appearances. The combination is so stable that carboxyhæmoglobin will remain unchanged in the blood for a long time. The author has specimens which have remained unaltered, in ordinary bottles, for many years, and which give the characteristic spectra and chemical reactions of carboxyhæmoglobin.

Another hæmoglobin derivative, **melanin,** occurs in the form of brown or black pigment granules in the blood plasma, and is generally due to the destruction of the erythrocytes by parasites. It is chiefly associated with malarial and relapsing fever. It is probably an iron-free body. It is often taken up by the leucocytes.

The richness of the individual erythrocyte in hæmoglobin is known as the **colour index**. The colour index is expressed as a fraction of which the percentage of hæmoglobin is the numerator and the percentage of erythrocytes the denominator. This is accepted as 1 in normal blood. To obtain this the percentage of hæmoglobin is estimated, and the number of erythrocytes in a definite volume are counted. Normally the hæmoglobin percentage is considered as 100, and the number of erythrocytes per cubic millimetre (i.e. 5,000,000) as 100. To estimate the colour index of the erythrocyte, the hæmoglobin percentage is divided by the erythrocyte percentage. For example, in normal blood.

Hæmoglobin =100 per cent.
Erythrocytes =100 per cent. (5,000,000).
100 ÷ 100 =1 normal colour index.

$$\text{or } \frac{\% \text{H}}{\% \text{E}} = \text{colour index}.$$

If % Hb be −, and % E. +, = colour index subnormal.
If % Hb be +, and E. −, = colour index is above normal.

As a rule the colour index is below normal in chlorosis and above it in pernicious anæmia.

ESTIMATION OF THE HÆMOGLOBIN

The estimation of the amount of hæmoglobin is one of the most important procedures in the clinical examination of the blood. There are several methods of doing it, each of which has special advocates who claim for it certain advantages. The methods used for clinical purposes provide only an estimate of the relative amount of hæmoglobin compared to the normal standard. From this, however, the absolute amount of hæmoglobin in a stated volume of blood may be calculated, if the normal hæmoglobin content be known. This has been stated above as 14 per cent. of the whole blood, and the amount in any given specimen may be calculated once the relative percentage has been determined by the hæmoglobinometer. Thus the percentage of hæmoglobin multiplied by 14 and divided by 100 gives the amount of hæmoglobin present in one hundred volumes of blood, or $\frac{(\%\text{Hb} \times 14)}{100}$ = grammes of Hæmoglobin in 100 grammes of blood.

From observations by Leichtenstern[1] the hæmoglobin content is greatest during the first week of life, and diminishes gradually to the age of puberty. From puberty to about the forty-fifth year it keeps at about the average, then gradually diminishes.

The relative percentages on clinical hæmoglobinometers have been calculated by Stierlin[2] taking Leichtenstern's observations as a basis, and are as follows—

New born	138·88
½–5 years	76·58
5–15 years	80·50
15–25 years	88·88
25–45 years	100·00
45–60 years	87·50

In healthy so-called full-blooded adults the hæmoglobin may even reach 110 or 120 per cent.

METHODS OF ESTIMATING THE PERCENTAGE OF HÆMOGLOBIN

The various instruments in use for this purpose are based upon a common principle of colour comparison. The specimen examined is compared with a standard colour which represents a known quantity or percentage of hæmoglobin.

For this purpose in the majority of methods employed the blood is first diluted.

Any one of the following instruments may be used for estimation purposes—

Gowers' hæmoglobinometer.
Oliver's hæmoglobinometer.

---

[1] Cited by Sahli, *Diagnostic Methods*, p. 616.

[2] *Arch. f. klin. Med.*, vol. xiv, 1889.

Von Fleischl's hæmoglobinometer.
Dare's hæmoglobinometer.
Sahli's hæmoglobinometer.
Tallquist's hæmoglobinometer.

Gowers' Hæmoglobinometer.—This instrument consists of two glass tubes, one of which contains a standard solution of picrocarmine in glycerine jelly of a colour equal to a 1 per cent. solution of normal blood. The other tube is graduated in percentage scale up to 120. Twenty cubic millimetres of normal blood diluted with distilled water to reach the scale at 100 should produce a solution of the same depth of colour as the standard. To obtain the necessary amount of blood a capillary pipette graduated to 10 and 20 cubic millimetres, with a piece of rubber tubing attached, is supplied for the purpose. A long fine glass pipette in addition is necessary, in order to dilute the blood gradually with distilled water.

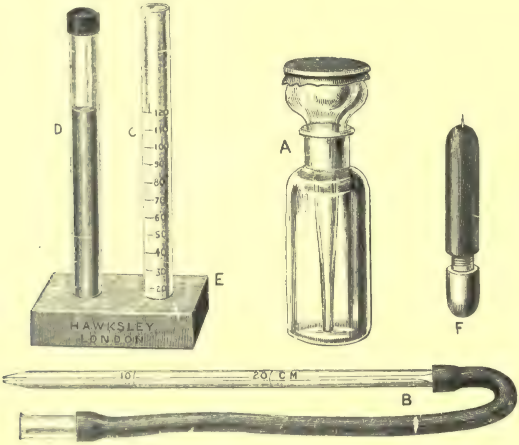

FIG. 2.—GOWERS' HÆMOGLOBINOMETER.

A—Bottle for distilled water with pipette stopper.
B—Capillary pipette and suction tube.
C—Graduated tube.
D—Tube containing standard colour.
E—India rubber stand for tubes *C* and *D*.
F—Lancet.

The standard and graduated tubes are flattened on two sides, and for comparison are placed in a rubber stand. White light should be used, and for this purpose it is desirable to compare the colours by looking through them against a sheet of white paper. When the percentage of hæmoglobin is very low, it is an advantage to place the tubes edge on to the sheet of paper.

Estimation of colouring matter by this instrument is quite simple. Twenty cubic millimetres of the blood are sucked up into the graduated pipette and blown into the graduated tube, distilled water is then drawn up to the same mark and blown into the graduated tube; by this means the capillary pipette is cleaned and no hæmoglobin lost. From the glass pipette stopper, into which distilled water has been drawn, add the latter drop by drop to the blood, mixing it each time, until the colour corresponds to that of the standard. Read off the scale, e.g. 60, on the graduated tube; this represents the percentage of hæmoglobin.

This instrument is accurate between 2 and 3 per cent., but below a reading of 10 per cent. it is very difficult to judge any difference in shade of colour with certainty.

Haldane-Gowers' Hæmoglobinometer. — In Haldane's modification of Gowers' instrument, instead of glycerine jelly tinted with picrocarmine, a 1 per cent. solution of blood containing the average percentage of hæmoglobin found in the blood of healthy men is used as a standard after saturation with carbon monoxide. It has an oxygen capacity of 18·5 per cent. as determined by the ferricyanide method.[1] The solution is hermetically sealed in a tube and is both definite and permanent. A second tube of 2·00 c.c. capacity is used for mixing the blood and water and is graduated up to 130 per cent. A capillary pipette for the blood holds 20 c.mm. when filled to the mark. A water-bottle with pipette stopper, a glass tube containing lancets, and a rubber tube with gas-burner attachment and which serves to deliver gas for saturating the diluted blood with CO, completes the instrument. If blood be drawn up to the mark on the capillary pipette and mixed with water in the graduated tube to reach the mark 100, a 1 per cent. solution of blood is obtained. This solution, if the blood be exactly normal, will, after saturation with CO or coal gas, have the same tint as the standard solution.

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[1] *Jour. Physiol.*, vol. xxv, 1900, p. 295.

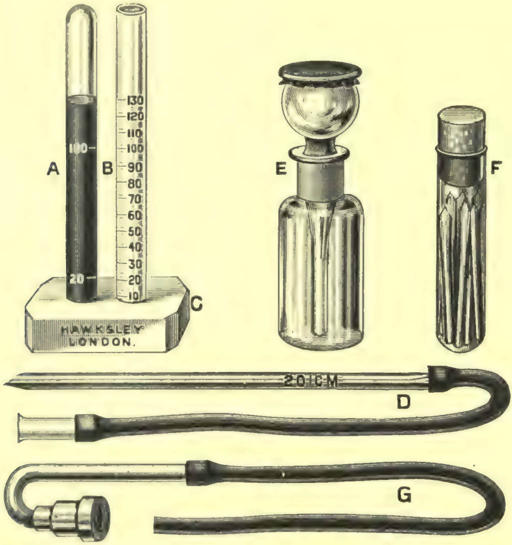

Fig. 3.—Haldane-Gowers' Hæmoglobinometer.

A—Glass tube containing blood solution of standard tint.
B—Graduated tube.
C—Rubber stand for tubes *A* and *B*.
D—Capillary pipette and suction tube; wires for cleaning the pipette are supplied.
E—Bottle with pipette stopper.
F—Glass tube holding six lancets.
G—Tube and cap for fixing over ordinary gas-burners.

If the blood contain more, or less, than the normal proportion of hæmoglobin, the dilution required to match the standard will be proportionately greater or less; the height of the liquid indicating the percentage of the normal.

Method of Use.—Sufficient water is first placed in the graduated tube to dilute the blood as far as safely possible. A puncture is then made in a finger or the lobe of an ear, and the capillary pipette (which must be clean and dry) at once filled to a little beyond the mark 20 from the drop of blood obtained. The point of the pipette is wiped, and dabbed on any convenient surface until the contained blood stands exactly at the mark. The blood is then gently blown out into the graduated tube, where it sinks ; the pipette is rinsed with the water in the graduated tube and withdrawn. The piece of rubber tube attached to a gas burner is now introduced into the graduated tube to near the level of the water, and gas allowed to pass for a few seconds. As the tube is withdrawn (with the gas still passing) the end is closed with the forefinger, and the liquid made to pass up and down in the tube—not violently shaken—at least a dozen times, so as to saturate the hæmoglobin with CO. During this manipulation the tube is held in a handkerchief, otherwise it will become heated and liquid will spurt out when the finger is withdrawn. Water is now added drop by drop with the pipette stopper, the tube after each addition being inverted, until the point is reached at which the tints of the liquids in the two tubes are just equal. In judging of the equality the tubes should be held against the light from the sky, or, if artificial light be used, from an opal glass shade. It is also absolutely necessary to transpose the tubes repeatedly : otherwise serious errors may arise. The level is read off on the graduated tube after half a minute has elapsed since the last drop added was mixed with the rest of the liquid by inverting the tube. The observation is repeated after the addition of another drop of water, and if necessary another, until the point is reached when the tints are again unequal. The true result is the mean of the readings giving equality. The error in any single determination ought not to exceed 1 per cent.

The result obtained is the percentage actually present of the average proportion of hæmoglobin in the blood of healthy adult men. The blood of healthy men, however, contains more hæmoglobin on an average than the blood of healthy women or children. Women give an average of 89 per cent., and children of 87 per cent. of the proportion in men. The results may be expressed in terms of oxygen capacity (the number of volumes of oxygen taken up in combination from air by 100 volumes of blood) if it be borne in mind that 100 per cent. on the scale corresponds to an oxygen capacity of 18·5.

The advantages of the modifications introduced into the original method of Gowers are: (1) that the standard solution is a definite one, so that an instrument can be verified at any time by making a determination with ox-blood of which the oxygen capacity has been determined by the ferricyanide method; (2) that the standard solution is permanent; (3) that the apparatus can be used with equal correctness by daylight and artificial light.

As coal-gas is not always available in examining the blood of patients, the instrument can always be supplied with an additional standard tube containing picro-carmine jelly, as in the original Gowers' Hæmoglobinometer. The picro-carmine jelly is standardized to correspond with blood of 18·5 per cent. oxygen capacity, but is liable to slow alteration on keeping. Its value in terms of the sealed tube of blood-solution should therefore be occasionally ascertained by determining the hæmoglobin in blood from the same person, first by the picrocarmine standard and afterwards by the sealed blood standard. The difference gives the percentage correction needed for the picrocarmine standard. The picrocarmine standard tube should be kept in the box, and not exposed unnecessarily to light.

Absolute accuracy as to size of tubes is not essential—since a correction can always be made by comparison with the sealed blood standard.

Oliver's Hæmoglobinometer.—This is an instrument based upon the principles of Lovibond's tintometer, and in which comparison is made with tinted glass standards. Light, reflected from a dead white surface of calcium sulphate is used for illumination, and the comparison is made by looking at the blood and tinted glass through a camera tube in order to cut off any extraneous light.

The instrument consists of a specially devised capillary pipette of a capacity of 5 c.mm. for measuring the blood, provided with a handle, a mixing pipette provided with a rubber nozzle which fits over the capillary pipette, and a rubber teat at the other end ; a small glass mixing cell with a white calcium sulphate base, in which to dilute the blood, and which when containing the measured quantity of blood and distilled water to the brim represents a blood dilution of 1 per cent. This is covered with a glass slip. The tinted standard glasses in the form of discs are arranged in two oblong frames, each disc is set on a white calcium sulphate base, and they are graduated in scale corresponding to hæmoglobin percentages from 10 to 120. Two sets, one for daylight, one for candle-light, are made. The latter is preferable for clinical work. A set of riders made of square tinted glass slips are provided for the purpose of estimating intermediate percentages between those of the standard discs. In the upper half of the scale they represent respectively 2½ and 5 per cent. of hæmoglobin, but twice that in the lower. Two sets of riders are provided, one for fine readings of 1 degree, the other for 2 degrees. The latter is sufficient for clinical purposes.

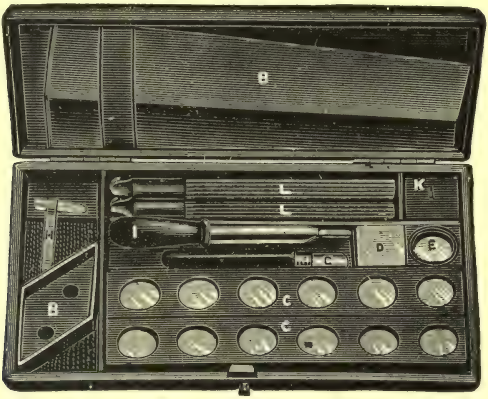

FIG. 4.—OLIVER'S HÆMOGLOBINOMETER.

B—Collapsible camera tube.
C—Graduated standard colour discs.
D—Riders.
E—Mixing cell.
G—Stilette.
H—Capillary measuring tube.
I—Mixing pipette.
K—Candle stand.
L—Candles.

To use the instrument, the capillary measuring tube is first cleaned and dried by passing through its bore a needle and thread. The proper quantity of blood is drawn into the tube by touching the drop, and ejected by means of the mixing pipette containing distilled water into the glass cell. It is then mixed by means of the handle of the capillary tube, and water is added until the cell is full. The cover slip is then applied. The cell is then placed opposite the standard disc to which it is approximate in colour. If its colour exactly corresponds with that of any single disc the reading may be taken at once. If not, it is placed opposite to the disc next below it in colour, and riders are added to complete the estimation, at the same time placing a square of white glass over the cell to compensate for the thickness of the rider.

The reading is made by candle-light, the candles used for Christmas decoration being quite suitable. The light should be placed at such a distance and in a position to ensure equal illumination of cell and disc. The examination is then made by looking at both cell and disc through the camera tube.

Von Fleischl's Hæmoglobinometer.—The principle of this instrument is the same as Oliver's. A volume of blood diluted with water to a definite strength is compared with a graduated wedge of tinted glass. The illumination is taken from a dead white calcium sulphate reflector, artificial light being used.

The instrument consists of a stand, which resembles the stage and lower half of a microscope stand. The stage has an aperture in the centre, through which light is thrown by means of the substage reflector. Beneath the stage a movable frame holding the wedge of coloured glass is fixed, and capable of horizontal movement by means of a rack and pinion. The frame bears a scale which is graduated to known percentages of hæmoglobin. Into the aperture of the stage is fitted a cylinder with a glass floor; it is divided by a vertical partition into two parts, one for the diluted blood and the other for distilled water, and the adjustment is so arranged that the latter should be superimposed on the wedge of coloured glass. Behind the opening for the cylinders is another through which the graduated scale may be read. Capillary pipettes with wire handles are supplied for measuring the blood, the capacity being such that when filled with normal blood and mixed with distilled water sufficient to fill one of the compartments of the cylinder the resultant colour will correspond to the glass wedge at the 100 mark. To use the instrument, the capillary pipette is cleaned and the requisite quantity of blood taken up by it; any blood on the outside of the tube should be wiped away. The blood is then ejected into the proper compartment of the cylinder, and rapidly and thoroughly mixed with distilled water by means of the wire handle. The other compartment should be filled with distilled water also. The colour of the blood solution is now compared with the glass wedge, the latter moved from side to side until the colours cor-

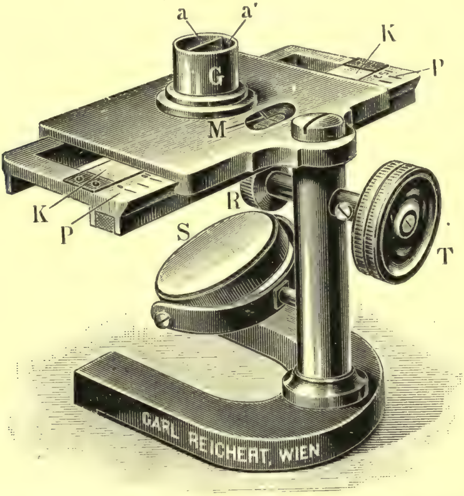

FIG. 5.—VON FLEISCHL'S HÆMOGLOBINOMETER.

G—Cylinder divided into two compartments; *a′* for distilled water, *a* for blood and water mixed.
K—Wedge of tinted glass.
P—Graduated scale.
M—Aperture and point for reading scale.
R, *T*—Rack and pinion for moving tinted glass.
S—Calcium sulphate reflector.

respond. Candle-light should be used for the purpose of illumination. A camera tube or roll of paper blackened on the inside may be used, as with Oliver's instrument. One should sit at one side of the instrument and use one eye for observation. When the colours of diluted blood and glass wedge correspond the percentage is read off by means of the scale.

A modification of this instrument has been devised by Miescher which enhances its accuracy.

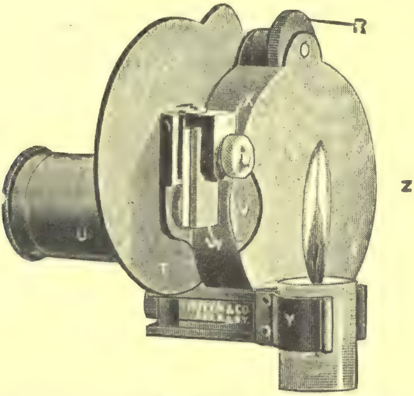

Fig 6.—Dare's Hæmoglobinometer.

R—Milled wheel for rotating coloured glass wedge.
T—Movable wing pivoted to case, and carrying
U—Telescoping camera tube.
V—Opening in prism case to illuminate coloured glass wedge.
W—Capillary cell for blood.
X—Clamp for capillary cell.
Y—Candle clip.
Z—Opposite rectangular opening in side of case through which to read hæmoglobin percentage.

Dare's Hæmoglobinometer.—This instrument resembles Von Fleischl's in that a coloured wedge of glass is used as a standard of comparison, but instead of a solution of blood the latter is used undiluted in a thin layer between two plates of glass, which are so arranged in a metal frame as to form a capillary chamber. One plate of the cell is made of clear glass, the other of opaque, through which the light is transmitted. Artificial light is used. The cell is fitted in the instrument behind a camera tube for observation ; and the wedge of coloured glass, which is in the form of a semicircle, is so arranged that it can be revolved behind an aperture adjacent to the capillary cell. The light is transmitted through the blood and coloured glass, at the same time illuminating both equally. When the colours of blood and tinted glass correspond the percentage is read off a graduated scale. The instrument is very convenient, and has the advantage of rapidity of manipulation, and the use of blood which has not to be diluted. The darker undiluted thin film of blood permits an easier comparison with a standard than does the lighter dilutions

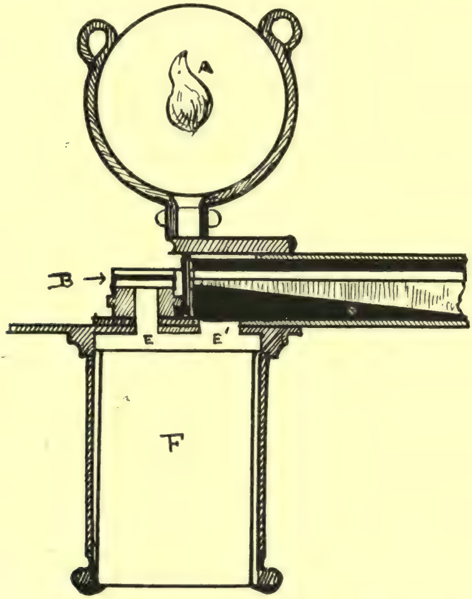

Fig. 7.—Horizontal Section of Dare's Hæmoglobinometer.

A—Candle.
B—Capillary blood cell.
C—Coloured glass wedge.
D—Opal glass disc behind coloured wedge.
E, E′—Apertures through which blood cell and coloured glass wedge are viewed respectively.
F—Telescoping camera tube.

necessary in other instruments. Errors of manipulation are practically avoided.

Sahlis' Hæmoglobinometer.—The principle of this instrument is modified from Gowers' hæmoglobinometer, and assures a permanency of the standard solution. The latter is made by the action of hydrochloric acid upon blood, so that hydrochlorate of hæmatin is produced, and this forms the standard colour. It corresponds to a 1 per cent. solution of normal blood. The blood to be examined is also acted upon with a definite solution of hydrochloric acid ($\frac{1}{10}$ normal) and the colour alters by reason of a similar formation of hydrochlorate of hæmatin varying in intensity according to its hæmoglobin content. Thus two solutions of the same colour are compared. The blood solution is mixed in a glass tube graduated to represent percentages of hæmoglobin. Twenty c.mm. of blood are drawn into a pipette similar to Gowers', and placed in the graduated tube, which has been filled to the mark 10 with the hydrochloric acid solution saturated with chloroform. When the mixture has become a clear dark-brown colour distilled water is added until it corresponds to the standard colour solution, and the percentage of hæmoglobin is read off on the scale. The two tubes are fitted into a black vulcanite holder to act as a light screen and backed by a ground glass plate as a light diffuser. The comparison is made by transmitted light, the result being the same whether it be artificial or daylight.

Tallquist's Hæmoglobinometer.—The principle of this method is based upon comparison of a colour scale on paper with the colour produced by a drop of blood upon white filter paper. The filter papers are arranged as leaves of a book in which is also bound the colour scale, this consists of ten sections of colour corresponding in range from 10 per cent. to 100 per cent. of hæmoglobin. A drop of blood having been soaked up by the filter paper, the colour produced is compared with the scale and the approximate percentage noted. By cutting out a disc from each of the tinted sections, the drop on the filter paper can be adjusted behind it and the comparison made more accurately. The examination is made by daylight. This simple method is very useful for rapid estimation at the bedside, but the reading must only be accepted as approximately correct.

Among other methods for the estimation of hæmoglobin may be mentioned the Hoppe-Seyler calorimetric double pipette, and the hæmatospectrophotometer of Vierordt and Hüfner, and Zangemeister's method in which the estimation is made by noting the thickness of the layer of blood solution required to correspond in colour to the standard.

The instruments described above are those more commonly in vogue and easy of manipulation.

An indirect method of hæmoglobin estimation introduced by Jolles depends upon the estimation of iron present in the blood. The iron content of hæmoglobin remaining constant, this method is accurate ; but it is limited to the examination of normal blood, and does not lend itself to pathological blood states. The instrument designed by Jolles, called the "ferrometer," is used by him for this purpose.

CHAPTER III

Spectroscopic Examination of Blood—Coagulation Time of the Blood—Estimation of the Coagulation Time—Estimation of Calcium Salts—Estimation of Alkalinity—Estimation of Specific Gravity—Volume of the Blood—Variations in the Volume of Blood and its Constituents—Estimation of the Viscosity of the Blood—Estimation of the Opsonic Content—Bacteriological Examination of the Blood—Widal's Reaction for Typhoid Fever—Demonstration of Fat in the Blood.

SPECTROSCOPIC EXAMINATION OF THE BLOOD

The spectroscope is particularly valuable in blood examinations in two ways : it may be used as a means of detecting the presence or absence of blood, or of differentiating between different blood states. For this purpose Browning's direct vision hand spectroscope is useful and convenient if sufficient blood be obtainable. If very minute quantities only are available then recourse must be had to one of the different forms of micro-spectroscopes which are used with the microscope. The blood solution to be examined constitutes the microscopic object and the spectroscope is substituted for the eyepiece. Artificial light must be used, and the D line located by placing in the flame a platinum wire carrying a salt of sodium. The spectra of blood are characteristic, and are due to the presence of hæmoglobin and its derivatives.

Oxyhæmoglobin presents a spectrum with two absorption bands between the D and E Fraunhofer lines ; the first band commences at the D line and extends a short distance towards E, the second commences after a short interval and terminates at the E line ; it is about twice the breadth of the first.

Reduced hæmoglobin presents one broad band occupying almost the whole space between D and E but slightly to the left of them. Oxyhæmoglobin may be reduced artificially by the addition of ammonium sulphide, or Stoke's reagent, consisting of ferrous sulphate in weak tartaric acid solution rendered alkaline with ammonia.

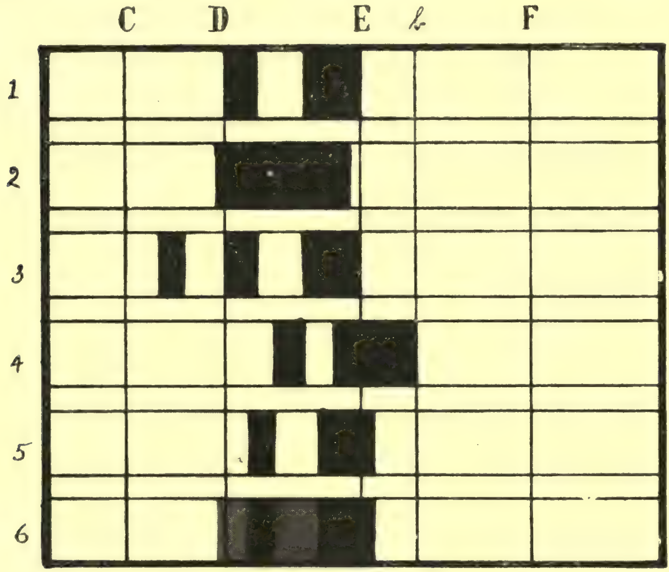

FIG. 8.—BLOOD SPECTRA.

1—Oxyhæmoglobin.
2—Reduced hæmoglobin.
3—Methæmoglobin.
4—Reduced hæmatin.
5—CO hæmoglobin.
6—CO hæmoglobin and reduced hæmoglobin.

Methæmoglobin presents two bands between D and E in the same position as those of oxyhæmoglobin, and a third band between C and D but nearer to the former; this band is the most pronounced of the three. A fourth band also occurs to the left of F.

Acid hæmatin is formed when hæmoglobin is acted upon by acids. It presents a spectrum with a band between D and E, commencing a short distance from D and ending at E, also a narrower band commencing at C and extending for a short distance towards D. It is a difficult spectrum to obtain.

Alkaline hæmatin, produced by the action of alkalies upon hæmoglobin, presents a single band between C and D and nearer to the D line. It is more difficult to obtain than the spectrum of acid hæmatin.

Reduced hæmatin, which gives the most pronounced of all blood spectra, is fomred when either of these last two derivatives is acted upon by ammonium sulphide in the presence of an alkali. It presents a dark absorption band between D and E and a broad but paler band commencing near the E line and extending to the *b* line.

Carboxyhæmoglobin, the combination of carbon-monoxide and hæmoglobin yields a spectrum which is very characteristic with two bands similar to those of oxyhæmoglobin but nearer to the violet end. Their position should be assured by accurate measurement and comparison with a spectrum of oxyhæmoglobin. Carboxyhæmoglobin is irreducible with ammonium sulphide or Stoke's fluid; the bands remain unaltered.

COAGULATION TIME OF THE BLOOD

The estimation of the rapidity of the coagulation of the blood includes alterations of viscosity and fibrin formation as these are inseparably connected with clot formation.

It is becoming more and more evident that this procedure is necessary in the clinical examination of the blood; for not only has it been established that the coagulation rate varies in different diseases and blood states, but it opens a field for investigation relative to the exercise of therapeutic measures. The process of coagulation is brought about through the splitting up of the soluble fibrinogen by the action of a ferment, thrombin, which is absent from the blood until it is shed, or some injury has occurred to a vessel wall, but which is produced by the interaction of a nucleo-proteid known as pro-thrombin supposed to be derived from the leucocytes, blood-platelets, or both, with the calcium salts of the blood.

Substances which act in the blood like thrombin are termed coagulins, and these also exist in the tissues. These tissue coagulins are varied; the vessel wall contains a tissue coagulin. In combination with blood coagulin the result is greater than the sum of their separate actions.

If blood be drawn into a tube or vessel which has been thoroughly oiled, and protected from dust, coagulation will be retarded for a considerable time; but the access of any foreign body will at once produce it. In the vessels the tissue coagulin is not set free until the cells are injured; they are supposed to be prevented from acting by the presence of so-called lipoids in the cell walls.

Reduction of the normal amount of calcium salts is said to retard coagulation; an increase to hasten it. Cold, extreme dilution with water, removal of oxygen and addition of carbonic acid gas, the addition of citric acid and citrates, retard coagulation. The process is hastened by agitating the blood or "whipping" it, by heat, by exposure to air and by the addition of tissue extracts. In relation to the latter is the retarding action of certain bacterial toxins, snake venom, leech extract, eel serum, peptones and certain colloid substances.

Some of them so act when injected into the body, but are inert in drawn blood. Some, such as snake venom and eel serum, also act in drawn blood.

The retardation in the body by these substances is preceded by an increase of coagulability. The fibrin ferment first produced is quickly destroyed, but how this happens is not settled. Anti-coagulins are normally present in the blood and tissues, and it is supposed that under certain conditions these may be formed in excess. In certain conditions associated with a hæmorrhagic diathesis, in the exanthemata, septicæmia, jaundice, and by the action of certain poisons, e.g. phosphorus, coagulation is retarded.

Former mention has been made of the deposit of fibrin in the examination of fresh blood. In normal blood this occurs in about three minutes after withdrawal, and is complete in about eight. An increase of the fibrin is called "hyperinosis," a decrease "hypinosis." The former is generally associated with febrile conditions and inflammations, and is a marked feature of pneumococcal infections. According to Matthews [1] it occurs in all suppurations.

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[1] *Amer. Journ. Physiol.*, 1899.

There is a direct relation between hyperinosis and leucocytosis except leukæmia,[1] although their co-appearance is not invariable. The amount of fibrin ferment in the blood depends upon the number of leucocytes, according to Stassano and Billon.[2] A peculiar condition described by Hayem[3] sometimes occurs in relation to coagulability. He found that in pernicious anæmia, purpura hæmorrhagica, malaria and some infectious diseases, that the serum failed to leave the clot or separated but slightly. He suggests that it may prove a means of distinguishing the forms of anæmia. The same condition gives rise to difficulty in obtaining anti-diphtheritic serum from the horse. Its significance awaits further investigation. The author has noticed this peculiarity in the blood in a case of myelogenous leukæmia.

ESTIMATION OF COAGULATION TIME

Up to the present it has been impossible to arrive at a definite standard coagulation time, because there are so many factors upon which this process depends, and which influence it in various ways. There is no doubt that coagulation differs in rapidity of onset in certain pathological conditions, and approximate estimates may prove helpful.

A simple method of estimating the coagulation time is by placing several drops of blood upon a glass slide, and at stated periods drawing across them a clean needle or bristle, noting the time after withdrawal before the first fibril of fibrin adheres to it. The size of the drops should be as near as possible alike. Vierordt[4] has devised a method of estimation, by taking into a capillary glass tube of 1 mm. diameter and several centimetres long, about 1 c.mm. of blood. A white horsehair previously boiled in alcohol and ether is passed through the tube from the other end into the drop at stated times and withdrawn;

[1] Pfeiffer, *Zeit. klin. Med.*, 1897, vol. xxxiii, p. 215.

[2] *Comptes rend. Soc. de Biol.*, 1903, vol. lv, p. 511.

[3] *Comptes rend. Soc. de Biol.*, 1901.

[4] *Archives der Heilk.*, 1878, vol. xix, p. 193.

as soon as coagulation commences the part of the white hair in the blood will become discoloured from adhering clot. By this method the coagulation time averages nine minutes.

Hayem[1] gauged the coagulation time by noticing the cessation of alteration in the level of blood in test tubes when tilted at intervals.

Milian[2] used drops of blood placed on numbered glass slides, these being tilted at intervals and the time noted when the contour of the drop ceased to change.

Bürker's[3] method is similar to the one first described above, but he dilutes the drop of blood on a slide with distilled water, and keeps it as nearly as possible at a constant temperature, using fine glass rods every half minute to test the drop for fibrin formation.

Sabrazes[4] and McGowan[5] use capillary tubes into which the blood is drawn. The tubes are broken and drawn apart at stated intervals and the thread of fibrin noted. Sabrazes keeps the tubes at a constant temperature, McGowan does not, but compares the result with the observer's blood at the same time.

Biffi[6] uses platinum loops of blood which, at stated intervals, are placed in water until one does not diffuse.

Buckmaster[7] also uses platinum loops of blood, which are rotated on their transverse axes in a warm chamber, and examined through a low power microscope. Rotation is carried on at intervals until the corpuscles cease to flow down the film, when it is in a vertical position. He found the average time to be $5\frac{3}{4}$ minutes at 31° C., and that it was shortened at higher and lengthened at lower temperatures.

Brodie and Russell's[8] Coagulometer.—This instrument consists of a chamber surrounded by a water jacket for temperature regulation. The floor of the chamber is covered by a layer of water, and its roof is formed by a movable glass slide in the centre, and from the under surface of which projects a truncated glass cone. Upon the under surface of this cone is placed the drop of blood for examination. Through one side of the water jacket a metal tube passes which contains another within it of glass, and tapering to a fine point; the point is directed upon the drop of blood from below. The process of coagulation is watched under a low power microscope, and a current of air is blown gently through the glass tube. This sets the corpuscles in motion, but when coagulation takes place they remain still. When they cease to move the time should be recorded, the edge of the drop should be used for the observation, as the stasis is more easily noted. This instrument gives fairly constant results at the same temperature.

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[1] *Du Sang*, Paris, 1889.

[2] Bull, *et mem. Soc. Med. d'Hôp.*, Paris, 1901; *Presse Med.*, 1904, vol. i, p. 202.

[3] *Archives f. ges Physiol.*, vol. cii, 1904, and cxviii, 1907.

[4] *Fol. Hœmat.*, 1904, p. 394; and 1906, p. 432.

[5] *Br. Med. Journ.*, 1907, vol. ii.

[6] *La Cronica Medica Ano.*, xxi, Num. 373, 1904.

[7] *The Morphology of Normal and Pathological Blood*, p. 212.

[8] *Journ. of Physiol.*, 1897, vol. xxi, p. 403.

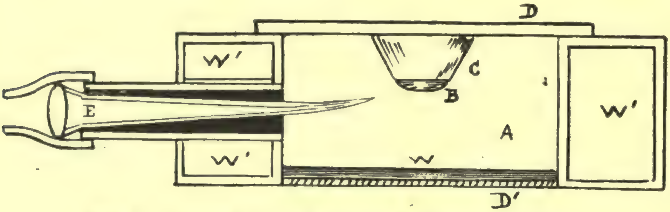

FIG. 9.—BRODIE AND RUSSELL'S COAGULOMETER.

A—Moist chamber.
B—Drop of blood.
C—Truncated cone.
D—Glass cover carrying cone.
D'—Glass plate.
E—Fine glass tube directing current of air.
W—Layer of water in moist chamber.
W'—Water jacket by which temperature is kept uniform.

Sir A. E. Wright's Coagulometer.[1]—This instrument consists of a metal water-tin surrounded by a leather jacket lined with flannel, which is so arranged as to form a series of pockets, one to carry a thermometer, the others to hold glass tubes. The latter are for the purpose of holding the blood which is drawn into them. A space should be

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[1] *British Med. Journ.*, 1894, vol. i, p. 237.

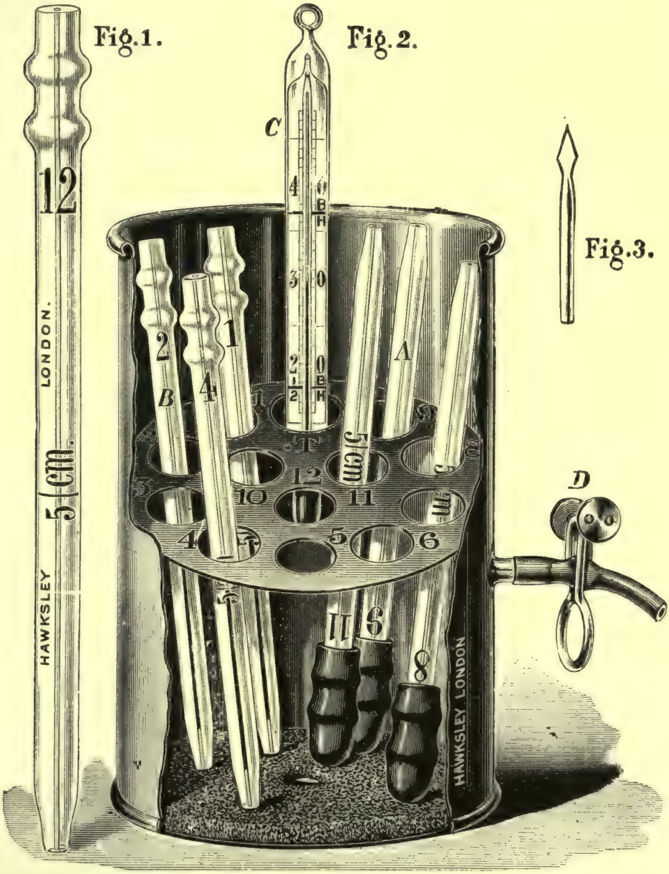

FIG. 10.—WRIGHT'S COAGULOMETER.

FIG. 1—A tube for blood.
FIG. 2—Metal water tin carrying twelve blood tubes.
  A—Empty tube capped and inverted to warm before use.
  B—Tubes containing blood, point downwards and un-capped.
  C—Thermometer.
  D—Water tap.
FIG. 3—Lancet.

left between both ends of the column of blood and the ends of the tube bore. The diameter of the tubes is 0·25 mm. For use the water can is filled with water at about body temperature, and the tubes placed in the pockets to warm them. The water must be kept at an equable temperature. When ready the tubes are withdrawn one by one at intervals of one minute, and partly filled with blood, taking care that the column in each is about the same size. They are numbered and replaced in the pockets. At stated intervals they are again withdrawn one by one and the blood blown out upon a sheet of white filter paper, so that the first sign of clotting is rendered noticeable. The tubes should be tested at stated periods until one is found from which the blood cannot be expelled ; the period when this happens subsequent to the filling of the tube may be taken as the coagulation time. A more accurate calculation is the mean between the shortest time taken for coagulation to be complete in a tube and the longest time recorded before the onset of coagulation in a tube.

The tubes are cleaned by passing a wire through them, washing with distilled water followed by alcohol and then ether.

Priestley Smith's Modification of Wright's Coagulometer.—Mr. Priestley Smith, of Birmingham, has introduced a modification of Wright's coagulometer. It consists of a series of eight glass tubes 25 mm. in length ; their lumen is 0·75 mm., and the wall is thick so as to be influenced as little as possible by the warmth of the operator's hand. The tubes are filled with blood from the ear and laid down side by side. After various intervals of time they are emptied under water in a white basin by means of a short rubber tube, into one end of which the glass tube is inserted, while the other end is closed by the finger and thumb ; compression of the rubber tube ejects the blood into the water. The object is to ascertain the length of time necessary for complete coagulation. When this has occurred the ejected blood presents a coherent and complete, or nearly complete, cast of the tube. The coagulation time, as is well known, is much influenced by temperature and on this point the author of the method has made a large number of investigations. They are not yet published, but he has supplied me with the following data—normal blood, tested by this instrument, shows on the average a coagulation time of eighteen minutes, when the thermometer in the box stands at 60° F. The time is shortened by about one minute for each degree of temperature above 60° up to 70° F., and it is lengthened by about one minute for each degree below 60° down to 55° F. The extent to which temperature modifies the coagulation time, when this is abnormal, has not been ascertained. The time varies also with the intake of water and food, and with other circumstances. The instrument is made by R. Bailey, Bennett's Hill, Birmingham.

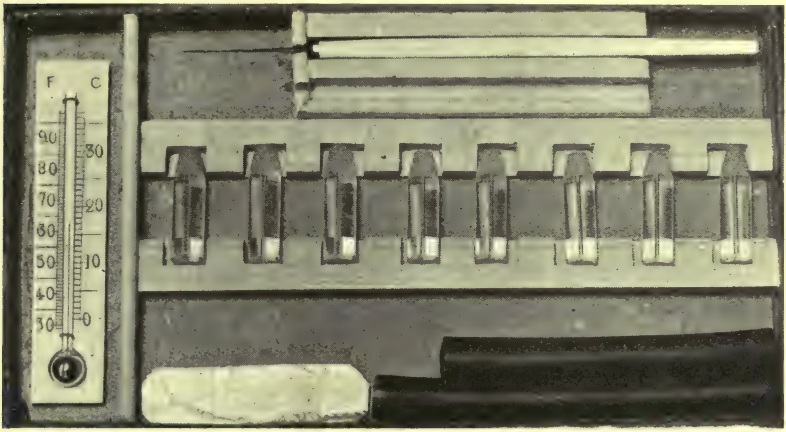

FIG. 11.—PRIESTLY SMITH'S MODIFICATION OF WRIGHT'S COAGULOMETER. (For description *vide* text.)

H. Fox [1] also uses calibrated tubes filled at intervals of half a minute and blown out into water until a worm-like clot forms. The method is practically the same as Priestley Smith's.

The Coaguloscope.—This instrument, devised by Mr. Priestley Smith, of Birmingham, and adapted for use by myself, is unique in its ingenuity. It enables the observer to watch the change of form which occurs in a small hemispherical mass of blood during coagulation. The blood is taken up from the ear or finger on the flat end of a small cylindrical ivory rod. A series of such rods thus capped with blood are placed upright and side by side in a metal bar. This latter slides into the bottom of a box, and through a magnifier in the top of the box the observer watches the changes which ensue. The top of the box has a double slope like the roof of a cottage, the one slope carrying the magnifier, the other having a circular aperture covered

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[1] *Lancet*, Jan. 11, 1908.

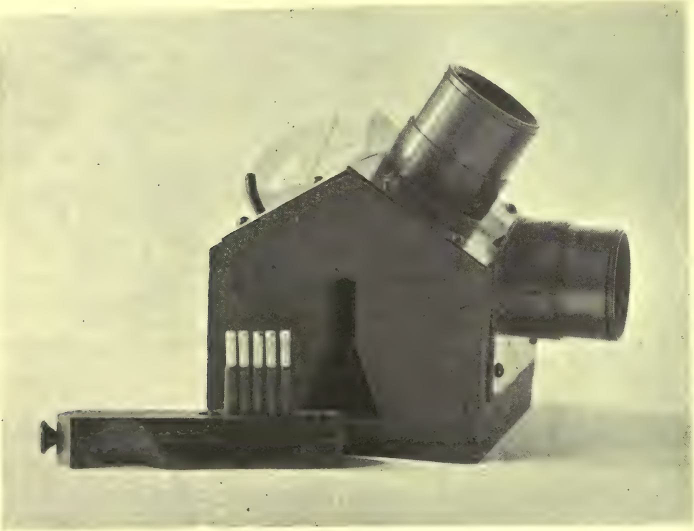

FIG. 12.—THE COAGULOSCOPE.
(For description *vide* text.)

by ground glass and a condensing lens for the purposes of illumination. The aperture is crossed diametrically by a black line, and affords an object whose image is seen brilliantly reflected in the convex surface of the drop of blood. The changes which occur in the image are an index to those in the form of the hemisphere of blood. At first the little blood dome has a perfectly smooth surface, and the reflected image occupies only its central region. Soon the image begins to enlarge, showing that the blood-surface is growing less convex, i.e. flatter ; then further changes follow which show that the blood-surface flattens completely over a considerable area, then becomes more or less deeply and widely cupped. The transverse line at a certain stage becomes blurred and a little later disappears, and the time occupied in reaching this stage is an index to the time of coagulation. But certain sources of error have to be reckoned with. Unequal quantities of blood will go through these changes in unequal times. Inequality may be discovered at the outset by the unequal size of the reflected images, a larger image indicating a flatter surface, and therefore a smaller amount of blood. One can measure the size of the image, as Helmoltz measured that of a corneal image, by doubling it by means of a prism, but this complicates the instrument and affords no great help. A further error may arise from the fact that the changes described depend to some extent on evaporation, and this will vary with the state of the atmosphere and the temperature. These difficulties led the originator of the instrument to abandon its use. I have endeavoured to overcome them, so far as evaporation and temperature are concerned, by abandoning the idea of a normal coagulation time and making in every case a comparison between the blood of the patient and that of a healthy person, tested at the same time and under the same conditions. I have usually employed my own. The result may be recorded as a coagulation index. Thus, if the coagulation time of the patient's blood is 12 minutes, and the normal blood 12 minutes, the coagulation index would be unity. If the patient's blood be 15 and the normal 12, then the index would be 0·8, expressed by the formula

$$\frac{\mathrm{N}}{\mathrm{P}} = \text{C.I. (Power of coagulation), or}$$

$$\frac{\text{Normal coagulation time}}{\text{Patient's coagulation time}} = \left\{\begin{array}{l}\text{Power of coagulation, or}\\ \quad\text{coagulation index.}\end{array}\right.$$

Addis's Coagulometer.[1]—T. Addis uses an instrument which he has devised and which is a modification of Brodie and Russell's method. The chamber in which the drop of blood is observed is circular and is filled with mineral oil kept at a regular temperature. The drop of blood is suspended in the oil from the truncated glass cone, and instead of air a current of oil is made to impinge tangentially upon it. Every precaution seems to have been taken by this observer to reduce experimental and technical errors to a minimum. The corpuscles rotate smoothly, quickly, and continuously, although the drop remains stationary. The movement is observed under a low power microscope. The coagulation time is measured from the moment when the finger is pricked to the time at which the flow of the corpuscles ceases. The corpuscles cease to rotate rather suddenly, and a laminated clot forms just before the flow stops. He considers that this instrument approaches as closely as possible the conditions obtaining within the blood vessels, the only foreign contact being the truncated glass cone from which the drop of blood is suspended. The average coagulation time he found to be 7½ minutes. From a large series of observations he draws the following conclusions.

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[1] The investigations of Dr. T. Addis were not published at the time the above account of them went to press. He very kindly placed his manuscript at my disposal. His work on blood coagulation

(1) There are four conditions which must be fulfilled by any method which is to yield reliable results : (*a*) There must be a uniform method of obtaining the blood. (*b*) The temperature must be the same during each experiment. (*c*) The amount of contact with foreign bodies must be equal, and always the same. (*d*) The end-point must be clear and definite and such as always to indicate the same degree of coagulation.

(2) The coagulation time is shortest at the body temperature; becoming gradually longer at temperatures above 40° C., and below 36° C.

(3) The coagulation is constant for the same individual at different times of the day, and even on different days.

will appear in the *Quarterly Journal of Experimental Physiology*, 1908, and on the effects of citric acid and calcium salts on the coagulation time in *The Quarterly Journal of Medicine*, Jan., 1909. (R. J. M. Buchanan.)

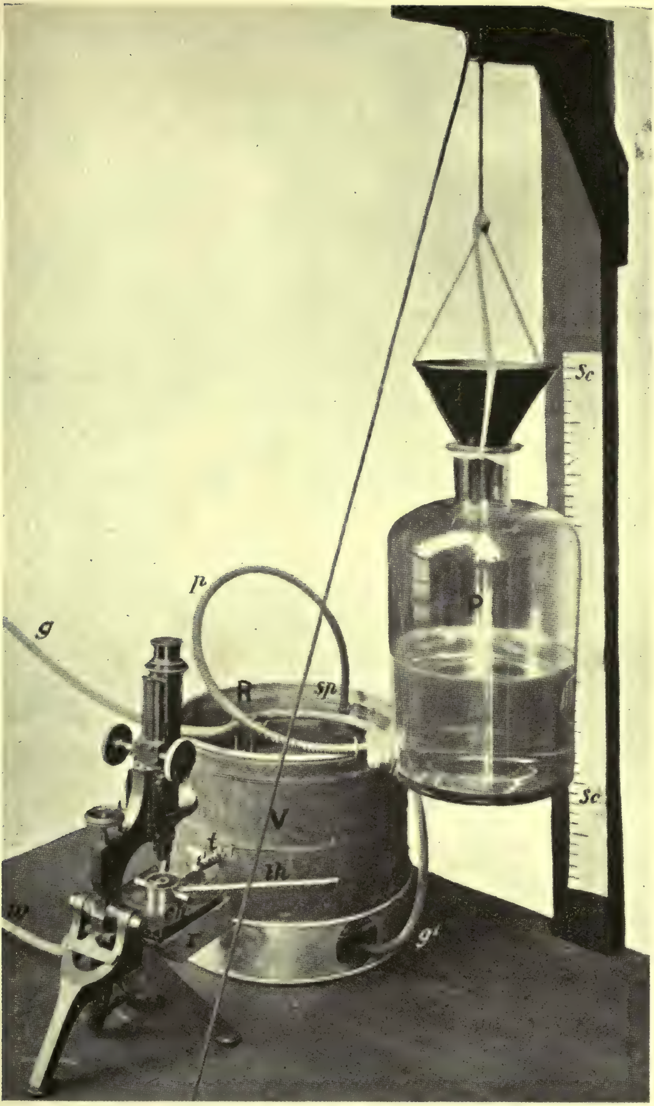

FIG. 13.—ADDIS'S COAGULOMETER.

P—Vessel containing filtered oil.
V—Water tank.
g, *R*, *g'*—Gas supply for heating the water.
p—Tube conducting oil by the spiral *sp* through warm water and by
t—to the Coagulometer.
ch—Coagulometer on microscope Stage.
r—Overflow oil tank.
th—Thermometer.
Sc—Scale on bracket recording height of oil.
w—Waste oil pipe.

(4) That the administration of salts of calcium or citric acid by the mouth has no effect upon the coagulation time, although it may produce increase or decrease respectively of the content of ionisable calcium in the blood.

(5) The amount of variation in the calcium content of the blood in vitro which is sufficient to lead to an appreciable change in its coagulation time, is greater than any variation which followed on the administration of calcium salts or citric acid by the mouth.

THE ESTIMATION OF CALCIUM SALTS IN THE BLOOD

A method has been introduced by Dr. W. Blair Bell, of Liverpool, and applied by him clinically for the estimation of calcium salts in the blood. With his kind permission I am enabled to describe the process in his own words. The illustration of normal oxalated blood on the hæmocytometer ruled squares is by Dr. Blair Bell.

Method as applied to blood.[1]—" A small capsule (Fig. 14 (1)) containing 250 c.mm. of oxalic acid (1 in 30 aqueous solution), sealed at the end of the stem, is taken to the bedside of the patient whose blood is to be examined. When all is ready the stem is broken at the mark *A*, care having been taken first to shake all the oxalic acid solution into the bulb, and the finger or lobe of the patient's ear pricked ; 100 c.mm. of blood are then drawn into a graduated

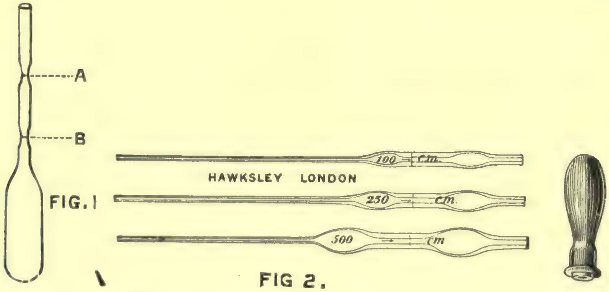

Fig. 14.—Pipettes and Capsule for Estimation of Calcium Salts.

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[1] Extracted from paper in *British Medical Journal*, April 20, 1907.

pipette (Fig. 14 (2)) and immediately emptied into the oxalic acid by passing the fine stem of the pipette down the neck of the capsule, and the mixture is well shaken for half a minute. The stem (or neck) of the capsule is then sealed. The contents on shaking become uniformly chocolate-coloured, and the blood is laked. After ten minutes this mixture is ready for further dilution, but if necessary the final processes may be left over for some hours, although the sooner they are carried out the better, for if left too long the heavy sediment may vitiate the result. When the examination is to be continued, the capsule, after the fluid has been well shaken down into the bulb, is broken

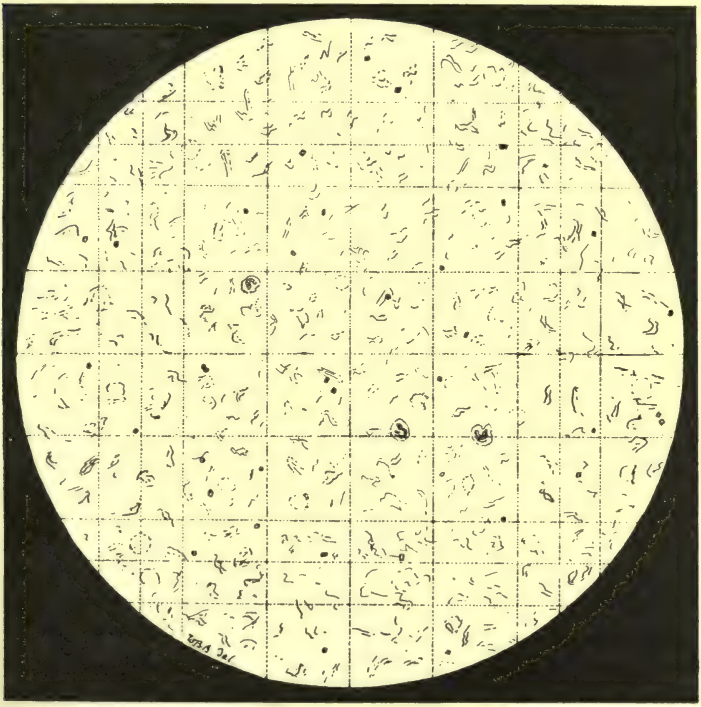

FIG. 15.—CALCIUM OXALATE CRYSTALS FROM NORMAL BLOOD.

(In addition to the crystals three leucocytes and debris of erythrocytes are shown on the hæmocytometer disc.)

at the mark *B*, and 250 c.mm. of the following mixture added to the oxalic acid and blood.

Glacial Acetic acid (1 per cent. aqueous solution.)	95 parts.
Glycerine	5 parts.

This, after being well mixed by shaking, is allowed to stand for another ten minutes, a rubber cap being placed over the broken end to prevent evaporation. The capsule is then thoroughly shaken, and 100 c.mm. are withdrawn and immediately mixed in another capsule with 500 c.mm. of distilled water previously placed there. It is of this last mixture that the examination is made.

A drop is placed on an ordinary Thoma-Zeiss hæmocytometer plate, *which must be carefully examined microscopically before each experiment to see that it is clean*, and the crystals of calcium oxalate are counted and noted for each square. At least 250 squares should be counted.

In ordinary individuals of middle life I find the normal number of crystals present averages about one per square, as a maximum. I therefore call the normal calcium index *one*.

The count is a perfectly easy matter, as the above dilutions result in a faintly-coloured solution affording a background against which the crystals show up well. A few hints as to details of technique are necessary.

Tap water must, of course, be avoided entirely in all washings throughout the whole of the procedures, and only reliable distilled water used. I have had several calculations entirely upset by impure distilled water. The greatest delicacy and accuracy are required in the measuring of the quantities; for to obtain an accurate estimate accurate dilutions are necessary. The special pipettes (Fig. 14 (2)) which I use are three in number, and are accurately marked for 100, 250 and 500 c.mm. for the various measurements required.[1]

An artificial light is much better than daylight for distinguishing the crystals, and the diaphragm must be almost closed. *The correct light is of the greatest importance for the easy recognition of the crystals.*

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[1] The apparatus required can all be obtained from Mr. Hawksley, 357, Oxford Street, London.

For counting, a magnification of 450 diameters, with an Abbé condenser, should be used. Only those crystals which can be definitely recognized should be counted. The fine adjustment should be moved to and fro in order to get a bright speck of light followed by a dark spot.

In blood solutions the crystals are very evenly distributed, are usually of uniform size, and do not lodge in the lines of the hæmocytometer plate; they are, too, until they have been on the plate for some little time, continually undergoing "Brownian" movements. This is especially the case if the plate is not completely covered with the drop. This movement is of great assistance in their recognition. Three or four minutes should be allowed to elapse after the drop has been put on the hæmocytometer plate, in order that the crystals may sink into a plane near the marked surface of the plate.

Recently, Dr. Blair Bell discusses the value of blood examinations,[1] and shows that a certain amount of judgment and knowledge is required in the interpretation. If correctly interpreted the blood index gives an index of the condition of the tissues in relation to calcium metabolism. It is probable that only the ionized calcium is estimated by this method, but since it is also probable that it is the calcium in this condition which takes part in the rapid tissue changes, the estimation of it is of considerable importance from a clinical point of view.

He has found that any medical or surgical lesion of sufficient severity lowers the blood index, owing to the determination of lime salts to the injured region, and he believes that calcium salts play a large part in all reparative processes.

In exophthalmic goitre he found the index low; in myxœdema the reverse. Consequently he came to the conclusion that the thyroid exercised a controlling influence in calcium metabolism, and he carried out experiments which supported his theories. Later experiments led him to believe that the other ductless glands also played a leading part in the metabolism of the lime salts. The diurnal variations were found to be very marked in normal individuals, and the calcium index was highest in the morning and after rest in bed.

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[1] *Liverpool Medico-Chirurgical Journal*, July, 1908; *British Med. Journ.* and the *Proc. Roy. Soc. Med.*, July, 1908.

He also demonstrated that in menstruation, pregnancy and lactation the calcium metabolism showed various important phenomena—it was, in fact, while working on these questions in connexion with the female genital functions that the other observations were made incidentally.

ESTIMATION OF ALKALINITY

The normal reaction of the blood may be acid or alkaline, according to the test solution used as an indicator. It is alkaline to litmus and methyl orange ; acid to phenolphthalein.[1]

Several methods have been devised for the purpose of estimating the alkalinity. They are based upon titration of blood or laked blood with solutions of tartaric acid of known strength, and testing with very sensitive test papers. The alkalinity is expressed in milligrammes of sodium hydrate per hundred cubic centimetres of blood.

Salkowski's [2] method is as follows. After adding a known quantity of sulphate of ammonia to the blood, it is allowed to stand under a globe and the ammonia set free taken up by sulphuric acid, it can then be estimated by titration.

Dare [3] titrates laked blood with tartaric acid solution until the spectrum of oxyhæmoglobin is replaced by that of methæmoglobin.

The following are results by—

Engel and Löwy	400–600 mg. NaHO	per	c.c.	blood.
Strauss	300–350 ,,		,,	,,
Waldvogel	350–400 ,,		,,	,,

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[1] B. Moore, *Proc. Roy. Soc.*, May, 1908.

[2] *Centralbl. f. die med. Wissenschaften*, 1898.

[3] *Proc. Path. Soc. of Philadel.*, April, 1903.

Wright's[1] method of estimating the alkalinity is useful because of the ease with which it may be carried out. Normal sulphuric acid is diluted to definite strengths; equal portions of serum and any strength of acid are mixed together by means of a pipette. Different strengths of acid are mixed with separate portions of the serum and each tested with very sensitive faintly red litmus paper until the mixture is neutral. The alkalinity is expressed in milligrammes of NaHO. The alkalinity may vary from $\frac{N}{25}$ to $\frac{N}{200}$ in disease, the normal being $\frac{N}{35}$

The alkalinity of the blood is due in part to alkaline salts which are diffusible, and in part combined with proteid. The diffusible salts are mostly present in the corpuscles, and the alkalinity depends largely upon their number. The alkalinity is lowered in severe anæmias, fevers, diabetes mellitus, uræmia and scurvy.

ESTIMATION OF SPECIFIC GRAVITY

There exists a close relationship between the specific gravity of the blood and its richness in corpuscles, but particularly the amount of hæmoglobin it contains. The latter relationship has been made use of as an indirect method of estimating the amount of colouring matter.

If the number of erythrocytes be diminished the specific gravity falls, the same with a diminution in the hæmoglobin content, the erythrocytes remaining normal.

Separated from each other, the erythrocytes are found mainly responsible for variations in the specific gravity, the plasma or serum remaining practically constant normally, but in pathological conditions, such as anæmias, renal disease and disorders of circulation, the specific gravity of the serum may be materially decreased.

In abnormal blood states such as leukæmia and lymphocythæmia, the specific gravity cannot be taken as an index to the hæmoglobin content.

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[1] *Lancet,* September 18, 1897.

The two methods most commonly used for estimating the specific gravity are known as the "**capillary pycnometicr**" and the other the "**ærometric.**"

The former, introduced by Schmalz,[1] is done by comparing the weights of equal volumes of blood and water. A capillary tube of 1·5 mm. diameter and 12 cm. long, and drawn out to a point at each end, washed with alcohol and ether, is dried and weighed; it is then filled with water and weighed; the water is removed, the tube dried again, filled with blood and weighed. The differences in weight of the empty tube and when filled with water or blood give their respective weights. The specific gravity will be represented by the weight of the volume of blood divided by the weight of the volume of water.

The "ærometric" or Hammerschlag's[2] method is a modification of Roy's and others, in which drops of blood are placed in fluids of known specific gravity until one is found in which the drop remains suspended. Instead of a mixture of glycerine and water which was formerly used for this purpose, Hammerschlag uses a mixture of chloroform and benzol. The drop of blood is placed in the mixture, and if it floats or sinks, benzol or chloroform are added respectively until the drop of blood remains suspended and stationary. When this happens the specific gravity of the mixture is taken with the ærometer or by specific gravity beads.

The specific gravity of the serum may be taken in the same way after centrifugalizing the clot from it.

Stintzing and Gumprecht[3] have suggested and practised a method for estimating the amount of dry residue of the blood, or hygromometry. The blood may be used in small quantities and is weighed in small vessels for the purpose. After drying for twenty-four hours at 65°–70° C., it is weighed again. In men the dry residue amounts to 21·6 per cent., in women to 19·8 per cent.

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[1] *Deutsch. Arch. f. klin. Med.*, vol. xlvii, p. 145, 1890.

[2] *Zeits. f. klin. Med.*, vol. xx, p. 444, 1892.

[3] *Deutsch. Arch. f. klin. Med.*, vol. xliii, 1894.

VOLUME OF THE BLOOD

Any of the methods which have been devised for the purpose of estimating the total volume of the blood have only afforded approximate results. In man it had been estimated at about one-thirteenth of the body weight. (More accurate estimations place it at about one-twentieth.)

Clinically it may be noticed that there are differences in the quantity of blood which may be obtained from a finger prick. In anæmic persons there may be a difficulty in obtaining specimens, while in the so-called "full-blooded" individuals the blood flows easily. The colour of the skin is an uncertain guide, because of its variation in thickness in different individuals, some being naturally pale, although their blood record may be normal; others may exhibit a pallor due to lack of colouring matter only. A method for estimating the blood volume suggested by Tarchanoff[1] is based upon the numerical comparison of the erythrocytes after artificial sweating, the loss of water having been measured. Counting the erythrocytes before and after transfusion of a known volume of blood has been advocated by Quincke,[2] but it does not offer any prospect of clinical value.

The method based upon the injection of a measured quantity of an antitoxin solution of known strength, which is allowed to circulate in the blood, and the latter then tested for its antitoxic power, offers many advantages and reliable results. For example, if 1 c.c. of (say) tetanus antitoxin be injected into the body, and the blood serum exhibits one three-thousandth part of the antitoxic power, the total volume of the blood will be 3,000 c.c., calculated after the relative volumes of serum and erythrocytes have been estimated.

Haldane and Smith[3] estimate the volume of blood by a colorimetric method after inhalation of CO gas. The individual is made to inhale 100 c.c. of CO gas, and the percentage of saturation of the blood with it is estimated. The colour of the blood is then compared with that of ox blood the capacity of absorption of CO gas by it being known, and the total amount of the individual's blood is calculated. Supposing the person's blood shows 20 per cent. saturation after inhaling 100 c.c. of CO gas, the whole blood would be fully saturated with 500 c.c. of CO. If the colour be the same as ox blood, 100 c.c. of which absorbs 20 c.c. of gas, then the person's blood contained 20 c.c. of CO gas in every 100 c.c., and to absorb 500 c.c. of the gas the total volume of the blood must be 2,500 c.c.

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[1] *Pfluger's Archives*, vol. xxiii.

[2] *Deutsch. Archiv. f. klin. Med.*, vol. xx.

[3] *Journ. Physiol.*, vols. xxii and xxv; *Trans. Path. Soc.*, Lond., vol. li.

They have thus estimated the total volume of blood at from one-thirtieth to one-sixteenth of the body weight.

VARIATIONS IN THE VOLUME OF THE BLOOD AND ITS CONSTITUENTS

Under this heading it will be convenient to consider the estimation of the relative volumes of corpuscles and plasma.

This is carried out by means of a centrifuge specially adapted for the purpose. There are many instruments which answer the purpose equally well, but the one most convenient and portable is Daland's Hæmatocrit.[1] A modification of this is the electric centrifuge, which is a great advantage in many ways. The instrument is composed of a specially arranged set of cog-wheels encased in a metal box, and driven by hand. The wheels are in connexion with an upright spindle, upon which is mounted and fixed by a bayonet lock, a metal frame which carries two capillary tubes. These tubes are graduated in hundredths. After filling the tubes with blood they are mounted in the frame, the outer ends being pressed against an india-rubber stopper by means of a spring. To prevent coagulation in the capillary tube it is advisable, as suggested by Köppe, to fill the tube with cedar oil and let the blood as it enters drive the cedar oil before it. This will prevent coagulation. After locking the frame upon the spindle, it is revolved by turning the handle, one turn producing one hundred and thirty revolutions. After about three minutes rapid centrifugalizing the operation is complete. The tube is examined and the level of the corpuscles read from the scale. In normal blood the corpuscles occupy one-half the entire length of the scale. To record the volume in percentages the scale reading is multiplied by two. As a recorder of the relative volumes of corpuscles and plasma the hæmatocrit gives very reliable results, but it cannot, as has been suggested, be used for the pur-

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[1] *Univ. Med. Magazine*, vol. iv, p. 85, 1891.

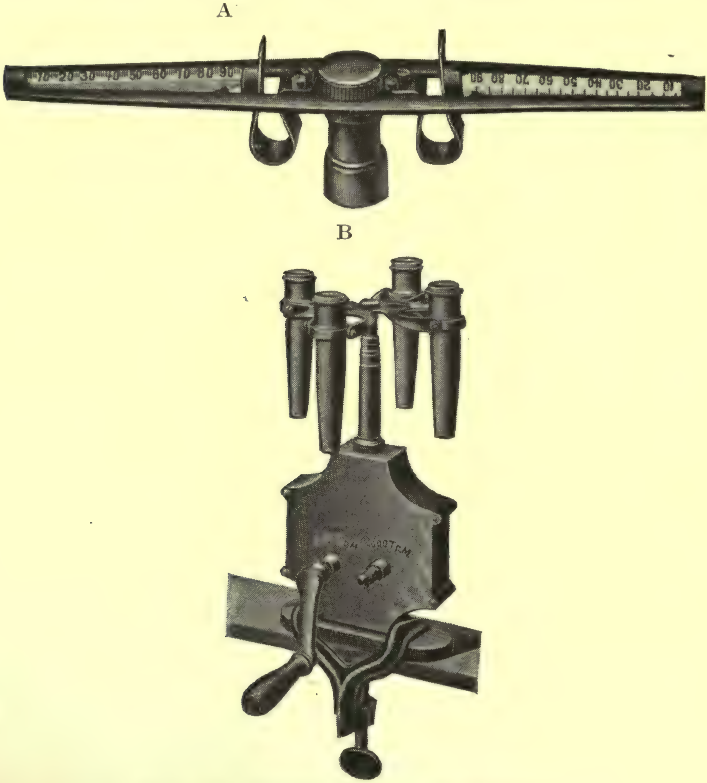

FIG. 16.—CENTRIFUGE AND HÆMATOCRIT.

A—Metal frame carrying graduated capillary tubes.
B—Centrifuge with fittings for four glass tubes which can be removed and replaced by metal frame.

pose of estimating the number of erythrocytes, because the record is rendered unreliable in many ways; for example, in pathological conditions the erythrocytes vary so much in size, and the presence of an increased number of leucocytes would easily affect the reading.

Capps,[1] however, has made use of the hæmatocrit in conjunction with the hæmocytometer to measure the volume of the individual erythrocyte. He calculates as follows. Supposing the hæmocytometer count records 40 per cent. of erythrocytes and the hæmatocrit 80 per cent. volume, the latter divided by the former gives a "volume index" of the erythrocytes as 2, or twice the normal. By this method Capps found that in pernicious anæmia the "volume index" is always increased, which corresponds to the microscopic picture of megalocythæmia. The secondary anæmias usually show a decrease in "volume index."

Within certain limits the blood supply to the tissues remains practically constant, owing to the adjustability of the vessels to the volume of their contents retaining a regular capillary pressure. Variations of the blood volume as a whole or certain of its constituents may take place.

In the former the change is generally loss rather than gain; in the latter it may be absolute, more often relative. Any one of these conditions is usually transient, but in certain instances corpuscular loss may prove permanent and progressive.

Whether any true natural increase of the total blood volume, or plethora, is ever present, is difficult to decide. No normal standard has been settled, and the accurate estimation of the total blood volume relative to body weight is no easy matter.

As a transient condition it may be said to occur after transfusion of blood, or mechanical distribution from pressure upon a limb by an elastic bandage.

A positive and relative increase of the fluid constituents may occur. The former has been designated serous plethora, the latter hydræmia; they are very similar, and occur under similar conditions, such as renal inefficiency and cardiac diseases or by transfusion of fluids. In hydræmia, however, there may be purely a relative increase of fluid constituents from an absolute loss of corpuscles. Such may arise as a pathological process, or follow a hæmorrhage, the fluid loss being made up again before the corpuscular.

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[1] *Journ. Med. Research,* vol. x, 3, Boston, 1903.

In hydræmic conditions the specific gravity of the blood is diminished. A loss of total blood volume, or oligæmia, is the usual result of hæmorrhage. It is as a rule transient, and the return to normal is preceded by hydræmia.

A relative diminution of fluids, or increase of erythrocytes, is the opposite condition to hydræmia, and has been termed anhydræmia. It generally follows a rapid and profuse discharge of liquids from the body, generally from the intestines, through the kidneys or by the skin; it may also occur during rapid serous effusions and states of high arterial tension (Oliver).

By the use of the carbon monoxide method, Lorrain Smith[1] has revealed facts in reference to the blood volume in disease which are of the utmost importance to clinical hæmatology. In chlorosis he demonstrates that the volume of blood is actually increased in proportion to the severity of the disease, the hæmoglobin content remaining normal, and the number of erythrocytes being absolutely increased. The leucocytes are also increased.

In pernicious anæmia the total hæmoglobin is diminished with the severity; the volume may be increased or diminished.

The volume of blood in post-hæmorrhagic anæmia is altered very little, and remains practically normal.

In some forms of valvular disease of heart with cyanosis, the volume, erythrocytes and hæmoglobin may all be increased.

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[1] *Journ. Physiol.*, vol. xxv, p. 333, 1900.

ESTIMATION OF THE VISCOSITY OF THE BLOOD

For this purpose a very simple and ingenious instrument has been devised by A. Denning and J. H. Watson,[1] and is to be obtained from Mr. Hawksley of Oxford Street, London. It consists of a J-shaped capillary tube, the long arm of which measures 6, and the short arm 2 centimetres in length. The upper end of the former is moulded so as to form a funnel-shaped receiver, and in the course of the short arm the tube is blown to form an elliptical bulb. The points where the capillary bore meets and leaves the bulb is marked on the glass. To use the instrument the lobe of the ear is pricked with a sharp pointed stilette (aseptic precautions being observed), and the instrument, which has been warmed to the body temperature, is held under the exuding drop of blood so that it flows into the receiver. The flow down the tube is carefully watched until it reaches the mark indicating the entrance to the

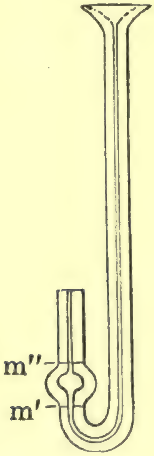

FIG. 17.—VISCOSIMETER.

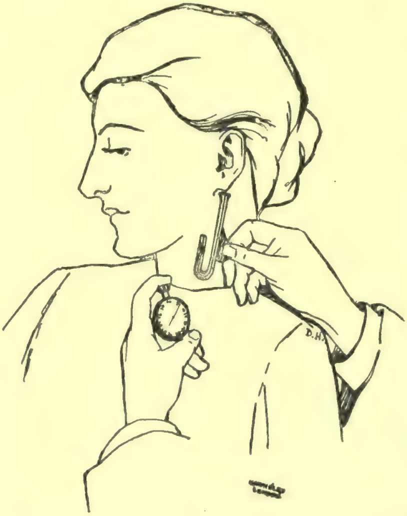

FIG. 18.—METHOD OF USING VISCOSIMETER.

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[1] *Lancet.* July 14, 1906 ; *Liverpool Med. Chir. Journ.*, July, 1906, p. 46.

bulb. The time is immediately noted on a stop watch, and again when the bulb is filled, when it is read off to the fraction of a second. The instrument is calibrated for water, the viscosity of which is known, and the time value is marked on the back of the tube.

The time value of the blood flow when compared with that of water gives the viscosity coefficient of the blood. In health it ranges between 4·8 and 5·6, that of water at the body temperature.

The study of the viscosity of the blood opens up a field of investigation which will prove of undoubted value in disease, especially in reference to polycythæmia, in which it is markedly raised, sometimes reaching twice the normal. It is also high in cases of cardiac disease, emphysema and bronchitis accompanied by cyanosis. That increased viscosity impedes circulation, points to the value of venesection as a therapeutic measure in the treatment of heart disease associated with cyanosis and polycythæmia. After using the viscosimeter it must be washed out with strong nitric acid, followed by water, alcohol and ether to ensure perfect cleanliness and dryness.[1]

ESTIMATION OF THE OPSONIC INDEX

Sir A. E. Wright[2] has shown that there exist in the blood stream substances which he has termed "**opsonins,**" and which have the power of preparing bacteria for phagocytic action on the part of the leucocytes. The opsonic content of an individual's serum can be measured, and upon this knowledge is based the treatment of infective processes by vaccines. To estimate the opsonic content of the serum one requires (*a*) leucocytes, washed free from plasma, (*b*) blood serum, (*c*) an emulsion of the micro-organism.

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[1] The author has found it difficult to cleanse the tubes with nitric acid, he prefers to use water or solution of citric acid or a citrate first followed by water, then the alcohol and ether. Nitric acid tends to coagulate the blood clinging to the tube. If any coagulum form, the author finds caustic soda or potash efficacious in its removal.

[2] *Pro. Roy. Soc.*, vol. lxxii, 1903 ; lxxiii, 1904 ; lxxiv, 1904. *Lancet*, Oct. 27, 1904.

The washed leucocytes are obtained by collecting blood into a centrifuge tube containing 0·5–1·5 per cent. of sodium citrate in normal saline solution to prevent clotting. The blood and solution are shaken up and then centrifuged; the supernatant fluid is then drawn off by means of a fine pipette, and the process repeated twice with normal saline solution. The blood will be separated into two layers, the lower consisting of red cells and the upper of a thin layer of leucocytes. The saline solution is now drawn off and the tube of blood is ready for use. The emulsion of the organism is made by thoroughly mixing in an agate mortar a minute portion of

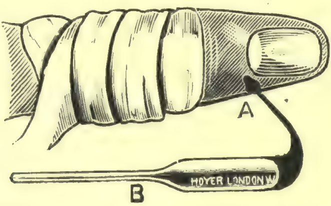

FIG. 19.—METHOD OF FILLING CURVED GLASS CAPSULE.
A—Curved end for receiving blood and to hang on centrifuge.
B—Ends of tube which is to be first warmed then sealed, and to which the blood will then pass before clotting.

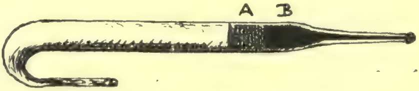

FIG. 20.—WRIGHT'S CURVED BLOOD CAPSULE, FILLED AND CENTRIFUGED.
A—Serum. *B*—Clot.

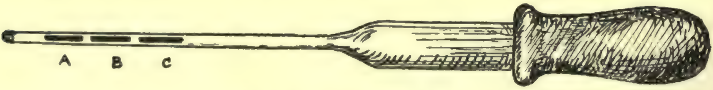

FIG. 21.—MIXING PIPETTE.
A—Bacilli. *B*—Leucocytes. *C*—Serum.
The spaces between *A*, *B*, *C*, contain air.

an agar growth, or of dried and dead tubercle bacilli, if these are to be used, with a sufficient quantity of normal saline solution to make a thin milky-looking emulsion which must be centrifuged to remove any clumps, and the upper fluid used. The serum is obtained from blood drawn from a finger prick, and collected in a Wright's curved glass capsule. After coagulation has taken place the blood is centrifuged and the clot separated from the serum.

Equal volumes of serum, leucocytes, and bacterial emulsion are drawn into a marked capillary pipette by means of a rubber teat attached to one end. These are then pressed out of the tube on to a glass slide and well mixed and drawn into the tube again, well away from the point, which is then sealed in the flame and the pipette and its content placed in an incubator at the body temperature for fifteen minutes. It is then removed, the end broken off, and the contents again pressed out on to a microscope slide, mixed, and smears made. When dried these are fixed and stained in any way suitable.

If the film be examined with the oil immersion after staining, it will be seen that, according to the opsonic content of the blood, phagocytosis will have taken place in greater or lesser degree.

The number of organisms in 100 leucocytes is counted, and this number divided by the number of leucocytes expresses the average per leucocyte or phagocytic count. Having found this for the normal serum, the same process is followed with serum from the person to be tested and the two compared. Assuming the normal index to be 1, the opsonic index of the person to be tested is expressed by the following formula—

$$\frac{\text{Patient's phagocytic count}}{\text{Normal phagocytic count}} = \text{Patient's opsonic index}$$

Thus : Patient's serum + leucocytes + organism = 5 per leucocyte.

Normal serum + leucocytes + organism = 10 per leucocyte.

Then $\frac{5}{10} = 0{\cdot}5$, the patient's opsonic index.

The opsonic index which may be found above or below normal, as the case may be, should be recorded on a chart and comparisons drawn from time to time. By inoculation of a person with a vaccine composed of dead organisms sterilized at 60° C. for an hour, an increase of opsonins takes place provided the dose be not too great. Immediately after the inoculation a fall in the opsonic content, or, as it is called, a "negative phase," takes place, followed by a rise or "positive phase," to a maximum or "high tide" of immunity which lasts for a certain time and then drops again or, "ebbs." If, however, a second inoculation be made during the negative phase, a further fall takes place, and by a series of negative phases brought about by inoculations in rapid succession, only harm will result and the resistance be lowered. It does not seem possible to produce a series of positive phases, so that the inoculations have to be spaced, taking the opsonic index as a guide, in order to prevent prolonged or serial negative phases, and to produce a succession of immunity high tides. The dose of vaccine used must be regulated by the degree of the negative phase, and so apportioned that this be as slight and of as short duration as possible, with the production of an early and high tide. In any case, it is always desirable to commence with a small dose.

BACTERIOLOGICAL EXAMINATION OF BLOOD

The demonstration of bacteria in blood may be done in films spread between cover-slips or on glass slides in the ordinary way. When obtaining the drop of blood for this purpose, the glass slips or slides must be scrupulously clean, and strict aseptic precautions carried out.

The dried films may be stained with Leishman's stain, which fixes the film at the same time. The colouration of the erythrocytes, however, interferes with the clearness of the stained preparation, so that some modification is necessary.

It is preferable to fix the film by heat, either by passing it three times through the flame of a Bunsen burner, or in a hot air oven heated to 120° C. It should then be washed in a 5 per cent. solution of acetic acid, which decolourizes the erythrocytes, again washed in distilled water, and the last traces of acid neutralized by exposing the film to the vapour of ammonia. The film may now be immersed in the stain to be used; any basic stain acts well, such as Löffler's methylene blue, or alcoholic solution of fuchsin.

It is only in exceptional instances that bacteria can be satisfactorily demonstated in blood films, e.g. anthrax. The more reliable way is to proceed by inoculation of culture media.

After careful sterilization of the skin, drops of blood may be carried in a platinum loop direct to the culture medium, such as bouillon or agar.

It is necessary to obtain a fair quantity of blood as the number of bacteria are usually few in number, hence it is preferable to withdraw the blood from a superficial vein into a sterilized syringe of about 10 c.c. capacity.

To ensure distension of the vein a ligature is applied round the limb, say the arm above the elbow, and the patient given an object to hold tight in the hand. The needle of the syringe is pushed into the vein in its longitudinal axis, and sufficient (say 5 c.c.) blood withdrawn. The blood should then be ejected in three separate portions into three flasks of bouillon, containing sufficient medium to dilute the blood several times, so as to lessen or overcome the bactericidal action of the serum, and so enhance the probability of successful culture. Each flask should contain 100 c.c. of bouillon.

In some cases it is not an easy matter to "needle" the vein; it may be collapsed from profound malnutrition of the patient, and difficult to distend from lack of blood; in such a case it may be necessary to resort to the operation of venesection, exercising strict aseptic precautions.

The further procedure of incubation and bacteriological examination of the inoculated media is beyond the province of this work.

WIDAL'S REACTION FOR TYPHOID FEVER[1]

In the blood of a person suffering from typhoid fever, bactericidal substances form, which act upon typhoid bacilli in such a way as to change their characteristic appearances as seen through the microscope. The reaction brings about a loss of motility of the bacilli and their precipitation in clumps.

The examination is made with the person's serum, and a twenty-four hours' culture of typhoid bacilli, which must be free from clumps, and the organisms must show active movements.

The blood from the patient is obtained from the cleansed finger, by means of a prick, and collected in a capillary tube, or pipette. A Wright's curved blood capsule is best of all, as a quantity of blood is obtained to give sufficient serum. After clotting has taken place the contents may be centrifugalized to obtain serum free from clot. With a platinum loop a drop of serum is placed upon a microscope slide, and next to it nine loopfuls of the typhoid bouillon culture. Instead of a platinum loop a marked pipette such as is used for opsonic work may be used. The blood serum and culture are mixed together and a cover-glass applied. The specimen is then examined with the microscope and the result observed. If the serum be taken from a person with typhoid fever, at the end of the first week, in some cases as early as the fourth day, the bacilli will be seen to lose their motility, and in a few minutes all movement may cease. They collect together in clumps, and in a typical reaction loss of movement and clumping is complete in thirty minutes or so. The weaker the serum the slower the reaction. If the reaction occurs in the ordinary way when greater dilution of the serum with the culture is made, e.g. 1 in 20, 50, it is regarded as more trustworthy. Instead of dilutions being made wholly with culture, normal saline solution may be used for part of them. The reaction occurs in 70 per cent. of cases from the fourth to the seventh day, in 80 per cent. from the eighth to the fourteenth, and in 90 per cent. during the third and fourth weeks.

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[1] *Bull. méd.*, 1896, vol. x; *Sem. méd.*, 1896, vol. xvi; *Lancet*, 1896, vol. ii.

It is absent altogether in about 5 to 10 per cent. of cases. Although a positive reaction, especially with high dilutions, is very strong evidence of typhoid fever, a negative reaction does not necessarily indicate the absence of the disease.

DEMONSTRATION OF FAT IN THE BLOOD

An excess of free fat in the blood, or lipæmia, occurs both under physiological and pathological conditions. It occurs in so many conditions that it thereby loses its clinical significance in disease. If abundant, as it is sometimes, particularly in diabetes, the blood may appear turbid and milky, especially noticeable in the serum after coagulation and centrifugalization.

It can be dissolved out with ether, and microscopically it may be easily recognized in the fresh specimen as highly refractile minute droplets, or in fixed films by their black reaction with osmic acid, or staining with Sudan III, by which they are coloured brick red.

CHAPTER IV

The Corpuscular Elements of the Blood—Erythrocytometry—Methods and Instruments—Leucocytometry—Enumeration of Blood Platelets.

THE CORPUSCULAR ELEMENTS OF THE BLOOD

Under this heading are included the red corpuscles or erythrocytes; the white corpuscles or leucocytes, which are true cell elements, and the blood platelets, the nature of which has not been finally determined.

Before proceeding to the consideration of the morphology of these elements, it will be advantageous to treat first of the methods used for their examination, in the following order—

Hæmocytometry
- (1) Erythrocytometry.
- (2) Leucocytometry.
- (3) Enumeration of blood-platelets.

(4) Preparation of specimens for microscopical examination.

ERYTHROCYTOMETRY

Various instruments are used for the purpose of counting the cells of the blood. The same instrument answers for both varieties of corpuscles.

Two of these, viz., the hæmocytometers of Thoma-Zeiss and Gowers, are arranged so that a number of cells in a known minute volume of diluted blood may be counted by the aid of a microscope.

The normal standard of comparison is the number of corpuscles contained in a cubic millimetre of blood, which is taken at 5,000,000 erythrocytes for males, 4,500,000 for females; the leucocytes averaging about 7,000—or a ratio of 1 white to 700 reds. In order to prevent alteration in the appearance of the corpuscles, certain solutions are necessary with which the blood is diluted.

Any of the following solutions may be used.

Gower's solution—An aqueous solution of sodium sulphate of sp. gr. 1025.

Toisson's—

Methyl violet, 5B	0·025 gm.
Sodium chloride	1 gm.
Sodium sulphate	8 gm.
Neutral glycerine	30 c.c.
Distilled water	160 c.c.

Hayem's—

Mercuric chloride	0·5 gm.
Sodium chloride	0·5 gm.
Sodium sulphate	2·5 gm.
Distilled water	100 c.c.

Sherrington's—

Methylene blue	0·1 gm.
Sodium chloride	1·2 gm.
Neutral potassium oxalate	1·2 gm.
Distilled water	300 c.c.

Acetic acid is useful in the diluting fluid for counting leucocytes as in the following formula—

Acetic acid	0·3 c.c.
Distilled water	100 c.c.

Methyl green or violet may be added in a sufficient quantity to stain the leucocytes without interfering with the illumination of the microscope. In addition to the staining action upon the leucocytes, this solution dissolves the red cells and so simplifies the process of counting.

Edington's solution.—The following solution is recommended by Alexander Edington [1] as useful for any kind of blood without any regard to its isotonic quality—

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[1] *Lancet*, July 13, 1907.

Neutral sodium citrate	7·5 gm.
Dahlia (Grübler's)	0·03 gm.
Formalin 40 per cent.	2·0 c.c.
Chloroform	3 drops
Distilled water	250 c.c.

Rub the stain down with a few drops of water, then mix with the remainder and add the formalin and sodium citrate. Leave it without filtering for a day or two. Keep it in a dark cool place and filter if necessary before using. In less than one minute all the corpuscles sink to focus in the counting chamber, and the refractive index is well maintained. In this solution blood may be transmitted for examination at a distance. Tubes for the purpose are made by Thomson, Skinner and Hamilton, 38, Sauchiehall Street, Glasgow.

Thoma-Zeiss Hæmocytometer. — This instrument consists of a graduated capillary pipette for collecting and mixing the blood, and a counting chamber. Two pipettes are usually provided, one for erythrocytes and one for leucocytes.

The pipette is pointed at the lower end and towards the upper is expanded into a bulb which contains a glass bead which serves as a mixer. Above the bulb the calibre is again narrowed and again expanded. A piece of india-rubber tubing is attached to the upper end.

The lumen of the pipette is graduated into tenths, and marked at 0·5 and 1 below the bulb, and 101 just above it. Blood drawn up to 0·5 on the tube, and then diluting fluid to 101, is diluted 200 times.

The counting chamber consists of a thick glass slide, carrying a circular glass disc ruled with lines enclosing 400 small squares each of an area of $\frac{1}{400}$ square mm. Surrounding this disc, with a narrow space or "moat" intervening, is a glass wall, which is exactly $\frac{1}{10}$ mm. higher than the upper surface of the disc. The whole is covered by a thin square of glass with perfectly plane surfaces. When the cover glass is on, the cubic contents of each small square is $\frac{1}{4000}$ cubic mm. The 400 small squares are divided into sets of 16 by horizontal and vertical lines dividing each fifth column of squares.

To use the instrument blood is drawn up the capillary tube to the mark 0·5; any adhering to the end is gently wiped off, and then the point of the pipette is plunged into the diluting fluid and the latter drawn to the mark 101. The pipette is then rotated in a horizontal position, or shaken with a finger closing each end, until the blood and diluent are well mixed. No bubble of air should be allowed to enter while filling the pipette.

To place a drop of the diluted blood upon the ruled disc, the portion contained in the capillary lumen of the pipette should be allowed to run out first. The next drop should be placed upon the ruled surface, and the cover slip adjusted. To make sure that the surfaces of the cover slip and wall surrounding the disc are in perfect apposition, Newton's coloured rings should be visible where they touch. It is not necessary that the whole area of the disc should be covered by the diluted blood, but the drop should touch both cover and disc. Great care should be exercised that none run over into the moat; should this happen, the cover slip must be removed and a fresh drop taken.

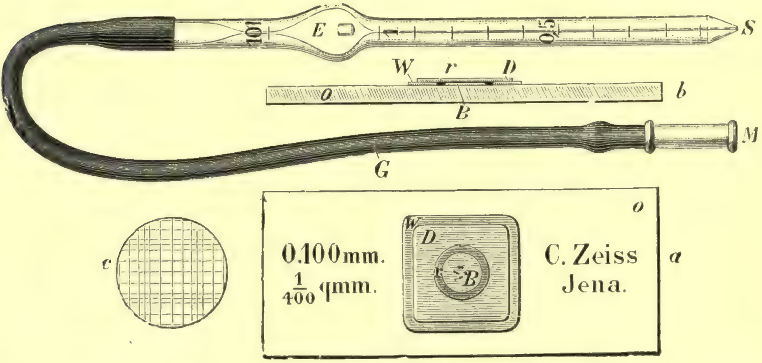

Fig. 22.—Thoma-Zeiss Hæmocytometer.

a—Slide of microscope with counting chamber. *b*—Same in section. *c*—Ruling of disc in squares. *W*—Wall. *B*—ruled discs. *D*—Cover-slip. *r*—Moat. *S*—Mixing pipette. *E*—Glass bead in bulb. *G*—Rubber tube. *M*—Mouthpiece.

It is essential that the counting chamber and cover be perfectly clean and dry before using them.

Having proceeded so far satisfactorily, the counting chamber is then placed on the microscope stage and left for a few minutes for the corpuscles to settle to the bottom. The ruled lines should be now focussed, using a $\frac{1}{6}$-in. objective and low eyepiece. The erythrocytes in the whole 400 squares should now be counted, commencing at the top left-hand square, counting each square horizontally from left to right, then the row below from right to left, and so on.

In addition to the erythrocytes enclosed in a square, those reclining on its upper and left boundary lines should be included.

Supposing the number of erythrocytes counted in the 400 squares be 2,800, then the average per square is 7; the blood, however, has been diluted 200 times and the cubic capacity of each square between cover and disc is $\frac{1}{4000}$ mm.; so that $7 \times 200 \times 4{,}000$ is the number of erythrocytes in a cubic mm., expressed in the following formula—

$$\frac{\overset{\text{Number of erythrocytes counted.}}{2{,}800} \times \overset{\text{Degree of dilution.}}{200} \times \overset{\text{Cubic contents of each square.}}{4{,}000}}{\text{Number of squares counted 400.}} = \left\{\begin{array}{l}\text{Number of}\\ \text{erythrocytes}\\ \text{per cubic mm.}\end{array}\right.$$

Gowers' Hæmocytometer.—This instrument consists of two pipettes, one of 995 c.mm., the other of 5 c.mm. capacity; a small glass vessel and stirring rod for mixing the blood and diluent, and a chamber for counting the erythrocytes. This is similar to the Thoma-Zeiss chamber, but the depth is $\frac{1}{5}$ mm. and the ruling forms squares with sides $\frac{1}{10}$ mm. The cubic contents of each square, when the counting chamber is covered with glass slip, measures $\frac{1}{500}$ of a cubic mm.

Any of the diluting fluids mentioned may be used. Gowers recommends an aqueous solution of sodium sulphate having a specific gravity of 1025. When using the instrument 995 c.mm. of diluent are measured with the large pipette and deposited in the mixing glass, 5 c.mm. of blood are measured with the fine pipette and immediately mixed with the diluent. This makes a blood dilution of 1 in 200. After mixing, a drop is transferred to the ruled squares of the counting chamber by means of the glass rod and covered with the glass slip, which is held in position by brass clips provided for the purpose. The examination and count is made with aid of the microscope.

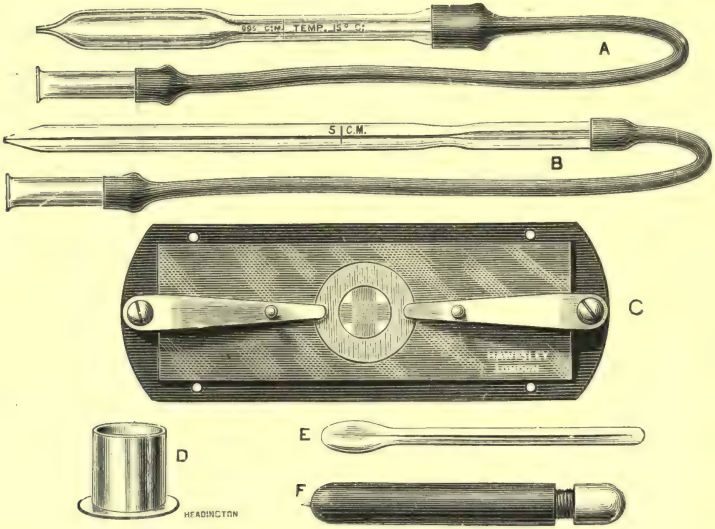

FIG. 23.—GOWERS' HÆMOCYTOMETER.

A—Pipette for diluent. *B*—Capillary pipette for blood. *C*—Counting chamber. *D*—Mixing vessel. *E*—Stirring rod. *F*—Stilette.

The formula for the calculation is—

$$\frac{\begin{matrix}\text{Number of erythro-}\\\text{cytes counted}\end{matrix} \times \overset{\text{Dilution.}}{200} \times \overset{\substack{\text{Cubic capacity}\\\text{of square.}}}{500}}{\text{Number of squares counted}} = \left\{\begin{matrix}\text{Number of}\\\text{erythrocytes}\\\text{per c.mm.}\end{matrix}\right.$$

Durham's modified Hæmocytometer.—In this instrument the Thoma-Zeiss counting chamber is used, the modification being in the form of the pipette.

Durham[1] devised self-filling capillary pipettes of 5 and 10 c.mm. in capacity. They are made of thick glass and mounted in another glass tube through a cork, the end of which is perforated and provided with a rubber teat having a perforation through one side.

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[1] *Edin. Med. Journ.*, October, 1897.

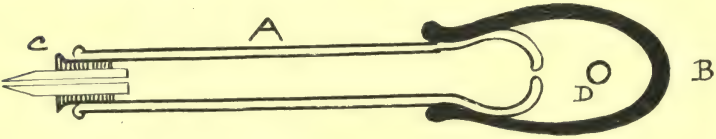

FIG. 24.—DURHAM'S PIPETTE.
A—Large tube. *B*—Rubber teat with aperture at *D*.
C—Capillary pipette for measuring blood mounted in cork.

Separate pipettes are used for measuring the diluent, and are graduated at 995 and 990 c.mm. and 495 and 490 c.mm. respectively. As mixing vessels small test tubes, each containing a small glass bead, are provided. As a guide to the dilution in any tube the beads may be of different colours. By using the proper capillary and large pipettes dilutions of 1 in 200, 1 in 100, and 1 in 50 may be obtained.

When using the instrument, the diluent is first measured into a mixing vessel, and then the capillary pipette chosen for the dilution desired. The drop of blood is allowed to flow into it until it is full, any excess wiped off, and the contents ejected into the mixing chamber by pressure on the rubber teat, with the thumb first over the perforation, but moved from it by a rotatory movement during the pressure, so that the teat reinflates through the hole in it without drawing any of the residual blood from the capillary tube. The point of the latter is now placed in the diluent and the pipette refills; pressure is again applied to the teat, and this process repeated until all the residual blood is washed into the diluent. By rotating the test tube rapidly between the palms of the hands, the blood and diluent is thoroughly mixed. A drop is then placed on the ruled disc of the counting chamber, and the enumeration made in the ordinary way.

Oliver's Hæmocytometer.—If a candle-light be looked at through a mixture of blood and a diluent such as Hayem's solution in a rectangular glass tube, providing the dilution

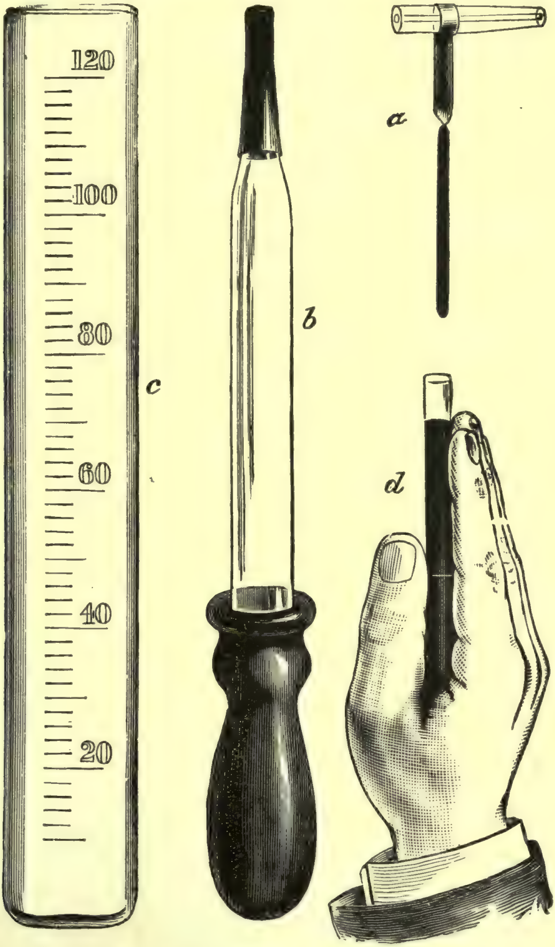

FIG. 25.—OLIVER'S HÆMOCYTOMETER.
a—Capillary pipette for measuring blood.
b—Medicine dropper for diluent.
c—Graduated glass tube.
d—Method of holding the graduated tube in order to view the bright line.

be of a certain strength, a bright horizontal line may be seen, which is made up of a series of small images of the flame due to the longitudinal striation of the glass. With normal blood the degree of dilution necessary in order to see the bright line is practically constant. The fewer the erythrocytes present in the blood, the less need it be diluted. Oliver's instrument consists of a rectangular glass tube 15 mm. by 5 mm. in cross section. It is graduated in hundredths from zero to 120 or 140. The mark 100 denotes the dilution of 10 c.mm. of normal blood necessary to allow the bright line to be seen. This represents 5,000,000 erythrocytes per c.mm., and each division of the scale is equivalent to 50,000. To use the instrument the small pipette of 10 c.mm. capacity is fixed in a handle and filled with blood; it is then inserted into a small rubber tube attached to a medicine dropper filled with Hayem's solution, and washed through into the graduated glass. The diluent is then added cautiously, and the tube well shaken. Holding the tube between the thumb and index finger and parallel to them, and on looking through it, keeping it close to the eye, and 10 feet from the candle, when the proper dilution has been arrived at the flame will be seen, appearing first at the edge of the long diameter. The bright line should be clearly visible across the narrow diameter of the tube for the dilution to be complete. The upper level of the fluid is read off the scale and the number of erythrocytes recorded.

Oliver recommends that a flame such as is given by a small Christmas candle is the best. The room must be dark with the exception of the light given by the candle flame.

A previous knowledge of the hæmoglobin content of the blood is a useful guide as to the extent of dilution which may be required.

No count can be made of the leucocytes by this instrument. A marked leucocytosis, as in leukæmia, or a profound anæmia, in which there are irregularities of shape and size of the erythrocytes, produce a considerable error.

LEUCOCYTOMETRY

The leucocytes may be counted in a similar way to the erythrocytes with a Thoma-Zeiss instrument. When by ordinary examination of the fresh specimen it is known that there is a marked increase in the number of leucocytes, the count may be made at the same time with the erythrocytes, or if preferred, without them, but using the same pipette.

As a general rule it is better to use the special pipette provided for the purpose. The procedure is the same, but the dilution is less, the pipette being graduated for 0·5, 1, to 11 c.mm. Taking blood to 0·5 and adding diluting fluid to the mark 11 will give a dilution of 1 : 20. If acetic acid solution be used, with or without the addition of a stain, the erythrocytes are destroyed and the leucocytes rendered more easily visible.

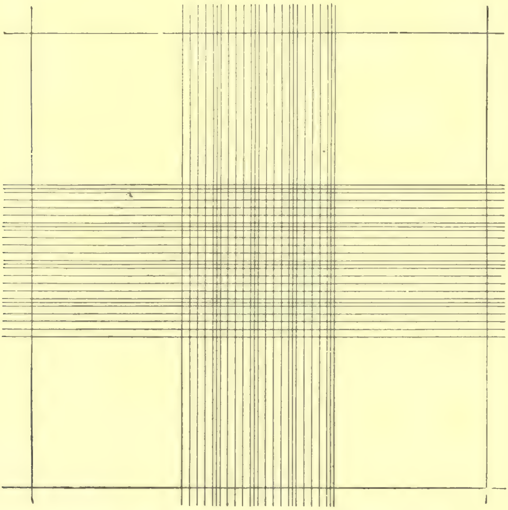

FIG. 26.—ZAPPERT RULED DISC.

Using the special pipette for leucocytes the formula is as follows—

$$\frac{\overset{\text{Number of leucocytes counted.}}{35} \times \overset{\text{Dilution.}}{20} \times \overset{\text{Cubic contents of each square.}}{4{,}000}}{\text{Number of squares counted, 400.}} = \left\{\begin{array}{l}\text{Number of leuco-}\\ \text{cytes per c.mm.}\\ (7{,}000).\end{array}\right.$$

It is an advantage when counting the leucocytes to use a Zappert ruled disc. This has upon it the same ruling of 400 small squares in the central square mm., but around it are ruled other eight squares of the same size, which if required may each be divided into four. Each large square represents $\frac{1}{10}$ c.mm. when the counting chamber is covered.

When using Zappert's chamber, the leucocytes in the central square mm. are counted with a low power, and then the other eight squares, the normal being about 35 per square; with a dilution of 1 : 20. The formula would be—

$$\frac{\overset{\text{Number of leucocytes counted.}}{315} \times \overset{\text{Dilution.}}{20} \times \overset{\text{Cubic contents of square.}}{10}}{\text{Number of squares counted, 9.}} = \left\{\begin{array}{l}\text{Number of leucocytes}\\ \text{per c.mm. (7,000).}\end{array}\right.$$

The process of counting may be simplified by using a diaphragm fitted into the eyepiece which has a central square aperture, the area of which, with the same lens, eyepiece and length of microscope barrel, exactly corresponds to the area of the magnified 400 small squares, i.e. 1 sq. mm. With the covered chamber, any area of it viewed through this diaphragm is equivalent to $\frac{1}{10}$ c.mm., so that once adjusted any number of squares may be counted without taking any note of the ruled lines. A movable stage is a great convenience. These diaphragms may be obtained with the instrument, or easily made from a piece of cardboard or thin metal.

Another method is to focus the ruled lines and then adjust the tube so that one of the parallel lines is situated at each side of the microscope field. Count the number of divisions across the field; e.g. 10 (each division being $\frac{1}{20}$ mm.), the diameter of the field would be $\frac{1}{2}$ mm. and the radius $\frac{1}{4}$ mm. The area of the field is obtained by multiplying the square of the radius by 3·1416, and the cubic contents by multiplying by the depth, i.e. $\frac{1}{10}$ c.mm.

Thus $\frac{1}{4} \times \frac{1}{4} \times \frac{1}{10} \times 3{\cdot}1416 = {\cdot}019635$, cubic contents of field.

Supposing the dilution used has been 1 : 20, and 300 leucocytes have been counted in 50 fields, then the final calculation is made by the following formula :

$$\frac{\overset{\text{Number of leucocytes counted.}}{300} \times \overset{\text{Dilution.}}{20}}{\overset{\text{Number of fields counted.}}{50} \times \overset{\text{Cubic contents of each field.}}{{\cdot}019635}} = \left\{ \begin{array}{l} \text{Number of leucocytes per} \\ \text{c.mm., 6,111.} \end{array} \right.$$

Stengel's method,[1] as recommended by W. G. Savage, is on similar lines to the last described. The Thoma-Zeiss erythrocyte pipette is used, the blood being drawn up to the 1·0 mark and diluted with one of the coloured diluting fluids to the 101 mark. The microscope is adjusted until the field of vision is spanned by an exact number of squares ; this number is called x. The leucocytes in a number of fields are counted and the average per field calculated $= y$. With a dilution of the blood 100 times the number of leucocytes per c.mm. is obtained by the formula—

$$\frac{56{,}000 \times y \times \text{dil.}}{11x^2.}$$

This formula may be used with any microscope and eyepiece.

Strong and Seligmann[2] count erythrocytes and leucocytes by using a measured quantity of blood diluted as desired, and allowing it to dry upon a slide, then mounting it in Canada balsam, and counting by means of a movable stage all the cells. From the amount taken and the dilution the number per c.mm. is calculated.

For the erythrocytes a fixing solution containing eosin is used, and for the leucocytes one containing methyl violet.

Leishman[3] dispenses with special apparatus with the exception of two pipettes, one a c.c. pipette graduated in hundredths, the other a capillary pipette 5 c.mm. capacity. With the large pipette 995 c.mm. of water are measured into a watch glass, and 5 c.mm. of blood added, making a dilution of 1 : 200. The erythrocytes become laked, but the leucocytes are unaffected. After thorough mixing, two separate volumes of 5 c.mm. are taken and deposited side by side on a microscope slide and allowed to dry. The specimen is then stained with Leishman's stain. A drop of Leishman's stain is allowed to evaporate upon a perfectly clean and well-polished cover glass. The film of stain is insoluble in water or cedar oil. It is ruled by means of a fine needle into parallel columns. The film of blood is covered with cedar oil, and the ruled glass placed over it. The count is then made with a $\frac{2}{3}$-in. lens, up one column and down the next. In this way both drops are examined, and the leucocytes in 10 c.mm. of diluted blood counted. By multiplying the result by 20, the number per c.mm. of undiluted blood is obtained. The amount of dilution may be adjusted to suit varied blood states.

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[1] *Lancet*, Sept., 1902.

[2] *Lancet*, May 23, 1903.

[3] *Lancet*, March 31, 1906.

ENUMERATION OF BLOOD PLATELETS

In the estimation of the number of blood platelets the use of a pipette has the disadvantage that the platelets have a tendency to adhere to the lumen. It is preferable to allow the blood, while flowing, to mix with the staining fluid used ; a drop of the mixture is then placed on a slide, covered, and the number of platelets counted in relation to the number of erythrocytes. The latter are then counted in the ordinary way with a hæmocytometer, and the number of platelets calculated. Thus : if the ratio of platelets to erythrocytes be 1 : 10 and the latter number 5,000,000 per c.mm., then the number of platelets would be 500,000 per c.mm. Various solutions may be used for receiving the blood.

Determann's Solutions.

0·9 per cent. aqueous solution of sodium chloride.
Methyl violet q.s.

or

Sodium chloride	1 gm.
Potassium bichromate	5 gm.
Distilled water to	100 c.c.

Hayem's Solutions.

Sodium chloride	1 gm.
Sodium sulphate	5 gm.
Excess of iodine in 5 per cent. aqueous solution of iodide of potassium	3·5 c.c.
Distilled water	200 c.c.

or

Methyl violet	1 gm.
0·75 per cent. aqueous solution sodium chloride	5000 c.c.

or

1 per cent. aqueous solution osmic acid	1 c.c.
0·75 per cent. aqueous sol. sodium chloride	2 c.c.

Bizzozero's Solution.

14 per cent. aqueous solution of magnesium sulphate.

Marcano's Solution.

Formaldehyde 40 per cent. in water	1 c.c.
Aqueous solution sodium sulphate (sp. gr. 1020)	100 c.c.

Affanasiew's Solution.

A 0·6 per cent. aqueous solution of sodium chloride and peptone with methyl violet.

Brodie and Russell's Solution.

Dahlia glycerine	1 c.c.
2 per cent. aqueous solution sodium chloride	1 c.c.

Jenner's Solution.

Glycerine	25 c.c.
Absolute alcohol	12·5 c.c.
Ammonium oxalate	1·0 gm.
Sodium chloride	1·5 gm.
Distilled water	62·5 c.c.

Dahlia (Grübler) "to saturation."

Any of the above fluids will prove satisfactory; preference must be given to those containing a staining reagent. Dahlia is an excellent stain for blood platelets. The ratio to erythrocytes may be obtained in dried films which have been subjected to double staining, e.g. methylene blue and eosin. It is necessary, when counting blood platelets, to select a preparation in which they are separate from one another. Much difficulty arises from their tendency to clump together in masses.

CLEANING PIPETTES

After using the pipettes for any of the above counts care should be taken that they are carefully and thoroughly cleaned and dried when finished with. The lumen of the pipettes may be cleaned by passing through them a bristle or piece of stout catgut to remove any material from the sides, then rinsing well with distilled water, followed by alcohol and ether. The latter may be removed rapidly by blowing air through the tube by means of a small rubber ball such as is used for working a spray producer.

CHAPTER V

Preparation of Specimens for Microscopical Examination—Films—Fixatives—Staining the Films—Stains and Methods of using them—Combined Fixation and Staining—Staining of Blood Platelets—Demonstration of Glycogen in the Blood—Mylius Reaction for Free Alkali—Examination of the Blood by means of Cut Sections.

PREPARATION OF SPECIMENS FOR MICROSCOPICAL EXAMINATION

The microscopical examination of the blood by means of fixed and stained specimens is indispensable for clinical purposes. It reveals important details which cannot be seen in the fresh preparation. The structure and shape of the erythrocytes and leucocytes may be studied, and the varieties of the latter differentiated. Not only may the characters of a normal blood-picture be studied, but also the departures obtaining in abnormal blood states.

The blood may be examined under two conditions: (*a*) The dried, fixed and stained blood film, which is most useful for clinical work; (*b*) stained sections of fixed blood, which are most desirable for cytological study.

PREPARATION OF FILMS

Films of blood may be spread upon cover slips or microscope slides. It is essential that the glass slips or slides should be perfectly clean and free from dust.

To clean them they may be washed in soap and water, rinsed in distilled water, then in ether, and kept in a glass vessel containing alcohol. When they are to be used they are removed from the alcohol and dried with a soft silk or linen handkerchief. Instead of keeping them in alcohol they may be dried and stored in a dust-proof glass vessel.

There are other methods of cleaning the glass, e.g. by washing in water containing alcohol and hydrochloric acid, then rinsing and passing through ether.

For slides it may be necessary to boil them in a solution of bichromate of potassium and sulphuric acid, and after rinsing in water to store them in alcohol; or wash them in dilute nitric acid, then in ammoniated water, finally pass them through distilled water to alcohol. By using clean glass, free from grease of any kind, the blood will spread more evenly and cling easily to it.

Cover glass films.—The drop of blood having been obtained by puncturing the skin in the ordinary way, the centre of a clean cover slip is gently applied to it; it is then placed drop downwards upon another cover glass, and the blood allowed to spread by capillarity and without pressure. The two slips are then pulled apart by gliding them gently one over the other.

When square slips are used it is most convenient to apply them angles to sides as in the diagram. A corner of the cover slip may be held in a forceps during the process, and to separate them the margins of the upper slip is lightly grasped by the fingers of the right hand. It often happens that the blood spreads more evenly on the under slip than the upper.

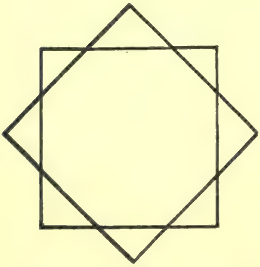

Fig. 27.—Position of Cover Slips for Spreading Film.

After separation, the films are allowed to dry in the air, but must be protected from dust.

Too large a drop must not be taken, for the film should be as thin as is compatible with perfect spreading. After the films have dried they may be placed in a small box and kept until required.

Films on glass slides.—The technique necessary to spread blood on glass slides is different from that just described.

The surface of the slide is applied to the drop of blood about half an inch from one end; it is then placed on a table with the drop upwards, and another slide is used as a spreader. Its narrow margin is allowed to touch the drop and while holding it at an angle of about 45° it is drawn gently along the horizontal slide from end to end, thus spreading the blood in a flat thin layer. Instead of drawing it the position of the spreader may be reversed and the film made by pushing the drop.

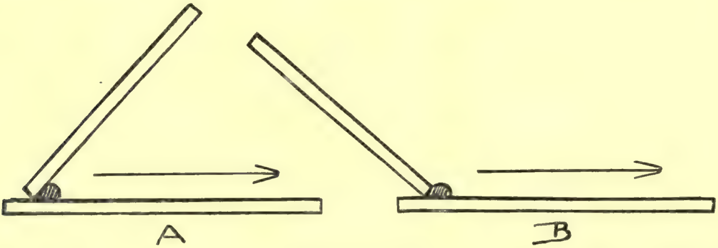

Fig. 28.—Method of Spreading the Blood Drop on a Glass Slide. *A*—By drawing. *B*—By pushing it with the narrow edge of another slide.

A cover slip, strip of cigarette paper or gutta-percha tissue may be used as a spreader; but if carefully handled, without undue pressure and with slides of even surface, the method described yields good and even films, without distorting the corpuscles. They are allowed to dry in the ordinary way. Slide films are preferable in many ways; there is less likelihood of their being broken, and they are easy of manipulation.

FIXING THE FILM

Before subjecting the film to any staining process it must be fixed; without this it would be washed from the slide in aqueous staining solutions. Fixation and staining may be carried out as separate processes or at the same time. The choice of a fixative depends upon the stain to be used subsequently. Fixation is obtained by the application of dry heat or chemical agents. Heat is most useful, and lends itself admirably to the majority of staining methods; for certain of them it is essential. I always use a hot air oven, heated by a Bunsen flame. A large number of films may be fixed at one time. Placed side by side in shallow cardboard trays and covered over in the oven, the temperature is raised to 120° C., at which it may remain from ten minutes to half an hour or more. A higher temperature than this even may be used with advantage, resulting in crisp specimens which stain brilliantly. The films may be placed in the oven and heated to 150° or 160° C., when the flame should be extinguished, and the oven allowed to cool to 30° C. before removing them. Instead of an oven, films may be fixed on a sheet of copper heated at one end over a Bunsen flame and the temperature recorded at the other. This method is not as satisfactory as the oven. For hurried observation the film may be fixed over a Bunsen or spirit flame, and then passed three or four times through it, but the fixation is rarely uniform and the results are uncertain.

Chemical fixatives.—Dried films may be fixed by placing them in absolute alcohol, or a mixture of equal parts of absolute alcohol and ether for ten or fifteen minutes. A saturated solution of perchloride of mercury in normal saline solution is an excellent fixative. Saturation must be assured by boiling, and crystals of the perchloride of mercury should deposit when the solution is cold. Films may be fixed either while moist or after drying. The mercury may be removed by washing in salt solution, followed by successive alcohols. The addition of a small quantity of iodine to the alcohol hastens the removal of the mercury.

The vapour of formol fixes well. A sponge soaked in formol is placed in a glass vessel, the films suspended in the vapour and the vessel covered. Cover slip films will adhere to the lid if it be moistened.

Benario's formol solution consists of—

10 per cent. aqueous solution of formol	1 c.c.
Absolute alcohol	10 c.c.

Films fix in a minute or two in this solution, and may be stained without drying.

Gulland recommends the following method—

Saturated solution of eosin in absolute alcohol	25 c.c.
Pure ether	25 c.c.
Perchloride of mercury 2 gm., absolute alcohol 10 c.c.	5 drops

Films are placed in this mixture without drying for three minutes; they may be left in for twenty-four hours if it be inconvenient to proceed to further staining at the time. After removal from the solution they are washed in water, and stained for a minute in saturated aqueous solution of methylene blue, washed again, dried and mounted if necessary.

Osmic acid.—2 per cent. solution is an excellent fixative. The film may be fixed in it either moist or dry.

Absolute methylic alcohol is a rapid and trustworthy fixative. Films may be placed in it for a few minutes, or it may be dropped upon the film, the latter being immediately covered over to prevent evaporation.

Absolute methylic alcohol is used as a solvent for certain stains. The process of fixation and staining are thus combined in one process.

STAINING THE FILM

For clinical purposes it is necessary to proceed in a way that will differentiate the nucleus and cytoplasm in any cell, and the varieties of cells from each other. To do this one resorts to double or even triple staining. The stains may be used successively or combined in a single solution. Further, the composite stain has selective affinities for definite elements of the cytoplasm, and by the choice of specific dyes these elements may be exhibited as desired. According to Erlich, the elective affinity of the protoplasm for stains depends upon two things, namely the chemical nature of the dye and the medium in which it is dissolved.

The so-called "granule" elements of the cytoplasm of leucocytes have been classified by Erlich as acid, basic and neutral, according to their affinity for acid, basic or neutral dyes. By an acid dye is meant a salt, the acid portion of which possesses the staining property, a basic dye the base, and a mixture of an acid and basic constitutes a so-called "neutral dye."

Eosin, orange *G*, acid fuchsin and indulin are examples of the acid dyes, methylene blue, fuchsin, safranin, gentian violet, methylene green and dahlia the basic dyes commonly used.

Hæmatoxylin still holds a deservedly popular place as a nuclear stain.

I have obtained excellent results with :—

Erlich's acid Hæmatoxylin—

Hæmatoxylin	1·5 gm.
Alcohol	100 c.c.

dissolve and mix with the following solution—

Saturated solution of ammonia alum in distilled water	100 c.c.
Glacial acetic acid	5 c.c.
Glycerine	100 c.c.

Drop some of this stain on the film and spread it ; over-staining does not easily take place. Wash well in distilled water and "blue" the nuclei with water containing a trace of lithium carbonate. Counter-stain in a 0·5 per cent. aqueous solution of eosin or saturated aqueous solution of orange G.

Delafield's Hæmatoxylin answers equally well.

Erlich's combined Hæmatoxylin with eosin or orange G—

Eosin (cryst.)	0·5 gm.
Absolute alcohol, distilled water, glycerine āā.	100 c.c.
Glacial acetic acid	10 c.c
Alum in excess.	

Several weeks are required for this solution to ripen. Films stain in from one half to two hours.

Eosin and **methylene blue,** used either successively or in combination, give excellent results.

Stain first in the following solution for half a minute—

Eosin	0·5 gm.
Alcohol	70 c.c.
Distilled water	30 c.c.

Wash thoroughly in distilled water, dry between filter paper and counter-stain in a saturated aqueous solution of methylene blue for a few seconds; wash again and dry. Löffler's methylene blue may be used.

Chenzinsky's Solution.

Concentrated aqueous solution of methylene blue	40 c.c.
Eosin 0·5 per cent. solution in 70 per cent. alcohol	20 c.c.
Distilled water	40 c.c.

This solution should be filtered before use.

Films should be stained for from six to twenty-four hours in an incubator.

Erlich's Solution.

1 per cent. aqueous solution of eosin	10 c.c.
Methyl alcohol	8 c.c.
Saturated aqueous solution of medicinal methylene blue	10 c.c.

After fixation by heat, staining is complete in from one to two minutes.

Da Costa's Solution.

Eosin (aqueous), to which sufficient water has been added for solution	0·5 gm.
Absolute alcohol	5·0 c.c.
Saturated aqueous solution of methylene blue	96 c.c.

Films are stained by the aid of gentle heat for one minute, left for three minutes to cool, and thoroughly washed in water.

Willebrandt's Solution.

0·5 per cent. solution of eosin in 70 per cent. alcohol.
Saturated aqueous solution of methylene blue.

Mix equal quantities. The fluid stains diffusely blue, but on the careful addition of acetic acid, 10 or 15 drops of a 1 per cent. solution to 50 c.c. of the stain, the eosin will act so as to produce a differential stain.

Filter before use, stain for ten minutes with the aid of heat, and wash in distilled water. Willebrandt holds that this method stains sharply all the granules and nuclei at the same time.

Prince's Stain.

Saturated aqueous solution of toluidin blue	24 c.c.
Saturated aqueous solution of acid fuchsin	1 c.c.
2 per cent. aqueous solution of eosin	2 c.c.

Mix in the order named, allow to settle and use the supernatant fluid.

Stain for one half to one minute; with old solution stain longer.

Goldhorn's Polychrome Methylene Blue.

Stain first in 0·1 per cent. aqueous solution of eosin; wash in distilled water, and stain in Goldhorn's polychrome methyl blue solution—

Methylene blue	2 gm.
Warm distilled water	300 c.c.

Dissolve and add, stirring all the time, 4 grammes of lithium carbonate; heat for fifteen minutes in a shallow water bath, stirring occasionally. Bottle the solution unfiltered. After a few days add 5 per cent. acetic acid solution until faintly alkaline.

Erlich's Triacid Stain.

Saturated solutions of orange G, acid fuchsin and methyl green are made with distilled water, and left standing to clear.

Mix as below.

Orange G solution	13–14 c.c.
Acid fuchsin solution	6–7 c.c.

Distilled water	15 c.c.
Absolute alcohol	15 c.c.
Methyl green solution	12·5 c.c.
Absolute alcohol	10 c.c.
Glycerine	10 c.c.

The mixture should be shaken while being made. It is ready for immediate use. Films should be stained for five minutes, washed in distilled water and dried. Specimens stain better when properly heated; if under-heated they stain too deeply with the fuchsin.

This stain acts best on specimens fixed by heat, but may be used with excellent results, as recommended by Michaelis, when films are fixed in Zenker's fluid and nitric acid for five minutes, washed and stained as above.

Zenker's fluid is made of—

Müller's fluid; or 2·5 per cent. aqueous solution of potassium bichromate	100 c.c.
Perchloride of mercury	5 gm.

To 100 c.c. add 5 c.c. glacial acetic acid and 5 c.c. nitric acid when required for use.

Israel-Pappenheim's Stain.

This stain is especially useful for determining the hæmoglobin content of the red corpuscles, and so is of great value in the examination of blood films in the anæmias. The structure and fate of the nucleus in nucleated erythrocytes can be clearly studied.

Stain first with Erlich's acid hæmatoxylin. Wash well, and then stain with the Israel-Pappenheim fluid for five minutes; wash and dry.

The solution consists of

Rose Bengale	6 gm.
Orange G	2 gm.
Aurantia	1 gm.

Mix the dry stains together and dissolve by the aid of gentle heat in sufficient of a solution made of

Distilled water	10 volumes
Glycerine	1 volume
Absolute alcohol	1 volume

Films should be fixed for ten minutes in absolute alcohol, they do not stain well if fixed by heat.

COMBINED FIXING AND STAINING METHODS

Jenner's Stain.

This stain is made by dissolving the precipitate formed by adding a solution of methylene blue to eosin, as follows—

(*a*)	Eosin aqueous yellow shade	1·25 gm.
	Distilled water	100 c.c.
(*b*)	Medicinal methylene blue	1 gm.
	Distilled water	100 c.c.

Mix equal parts of (*a*) and (*b*) adding the methylene blue solution to the eosin. Stir constantly and allow the precipitate to settle. Filter and dry the residue in an incubator; powder and wash it in distilled water; filter, dry in the air, and keep it in a tightly-stoppered bottle.

For staining make a solution of 0·5 gm. of the powder in 100 c.c. absolute methyl alcohol.

This solution is dropped on to the dried film and allowed to remain for five minutes, evaporation must be prevented by covering the film with a watch-glass or Petri dish. Wash for fifteen seconds in distilled water until the film appears pink, and dry in the air or with filter paper.

The stain gives the best results when the blue and eosin solutions in methyl alcohol are used as follows—

(*a*)	Water-soluble eosin, yellow shade	1 gm.
	Absolute methyl alcohol	100 c.c.
(*b*)	Medicinal methylene blue	1 gm.
	Absolute methyl alcohol	100 c.c.

Mix 10 c.c. of (*a*) with 12·5 c.c. of (*b*).

Stain for about five minutes, and wash in distilled water as above.

The eosinate of methyl blue may be obtained in the form of powder or "soloids," and ready for solution in the methyl alcohol, which should be done shortly before it is required for use.

Leishman's Stain.

This is a modification of Romanowsky's method of staining and is excellent for routine work.

The stain is made as follows—

(*a*) A 1 per cent. solution of medicinal methylene blue in distilled water is rendered alkaline by 0·5 per cent. solution of sodium carbonate, and heated at 65° C. for twelve hours. It is then allowed to stand at the temperature of the room for ten days.

(*b*) 1 per cent. solution of eosin (extra B, A, Grübler) in distilled water.

Equal parts are mixed, with stirring, and allowed to stand for from six to twelve hours. The resultant precipitate is collected on a filter, washed with distilled water, dried and powdered. The powder is used for staining as a 0·15 per cent. solution in absolute methyl alcohol (Merck's).

The powder may be obtained ready for use, as noted in Jenner's stain. Three or four drops of the stain are dropped on to the dried film and allowed to stand for one minute, no attempt being made to prevent evaporation as in Jenner's stain. Six to eight drops of distilled water are then added and mixed with the stain by means of a fine glass rod used with a stroking movement. The diluted stain is allowed to act for from five to ten minutes, and is then washed off with distilled water. A small quantity of the water is left on the film for another minute or so and it is then washed again and dried.

Wright's Modification of Leishman's Stain.

The method of staining introduced by J. H. Wright is somewhat similar to those of Jenner and Leishman.

To prepare the stain 0·5 gm. of sodium bicarbonate is dissolved in 100 c.c. of distilled water and 1 gm. of polychrome methylene blue added. This solution is sterilized in a steam sterilizer for an hour. It is then poured into a wide dish, and, while stirring, enough 1 in

1,000 solution of Grübler's yellow water-soluble eosin is added until the solution becomes purple, with a yellow metallic scum on the surface. This will require about 500 c.c. eosin solution to 100 c.c. of the alkaline methylene blue solution. A precipitate forms, which must be collected on a filter and dried without washing. To make the stain, 0·3 gm. of this powder is dissolved in 100 c.c. pure methylic alcohol, filter, and to 80 c.c. of the filtrate add 20 c.c. of pure methylic alcohol.

The blood film is covered with a few drops of the stain, which is allowed to act for a minute. Distilled water is then added until a greenish metallic scum appears on the surface, and it is left for two minutes. The film is then washed in distilled water until the film is yellowish pink. Dry with filter paper.

STAINING MAST CELLS

For this purpose Jenner's or Leishman's stain may be used, or by fixing and staining with a 10 per cent. solution of polychrome methylene blue in absolute methyl alcohol.

Erlich's Method.—Films fixed with alcohol may be stained with the solution recommended by Erlich for one or two hours.

Glacial acetic acid	12·5 c.c.
Absolute alcohol	50 c.c.
Distilled water	100 c.c.
Dahlia to saturation.	

After staining wash in distilled water and dry.

Turk's Method.

Films are fixed by heat or absolute methyl alcohol, then stained in

Methylene blue (medicinal Grübler) .	1 gm.
60 per cent. alcohol	100 c.c.

While staining the solution is warmed to 60° C., and allowed to cool.

The film is washed in distilled water and placed for half a minute in iodine solution 1 : 300; again washed and mounted in the following fluid—

Iodine	1 gm.
Potassium iodide	3 gm.
Distilled water	100 c.c.

Gum arabic q.s. to make a thick syrup.

With this stain the basophile granules in the mast cells and erythrocytes are stained black, polychromatophilic erythrocytes are dark green, nuclei pale blue.

Buchanan's Method.

Stain the fixed film in a saturated aqueous solution of methylene blue, or in Löffler's stain, wash in distilled water, dry with filter paper and mount in the following solution—

Iodine	1 gm.
Potassium iodide	3 gm.
Mucilage of acacia	50 c.c.
Distilled water	50 c.c.

With this method, the nuclei are stained mahogany brown, and the basophile granules dark brown to black. The erythrocytes are a deep orange colour in normal blood and ordinary anæmia, but in pernicious anæmia they are of a pale green colour.

STAINING BLOOD PLATELETS

Any of the staining solutions mentioned for the enumeration of blood platelets may be used.

In dried films excellent results are obtained with Leishman's or Jenner's stains. They respond to almost any basic stain in fixed films, especially dahlia, for which a formula has been given under mast cell staining. Being amphophilic in their affinities for dyes, they do not react well to double staining when solutions are used successively.

Either of the methods of staining with methylene blue and mounting in iodine-gum solution are applicable in fixed films. They respond to the processes for the demonstration of glycogen and alkali in the blood.

DEETJEN'S METHOD OF EXAMINING BLOOD PLATELETS

By this method the platelets are examined in fresh blood. A 2 per cent. solution of agar in distilled water is made by boiling, and filtered hot; to 100 c.c. of it is added 0·6 gm. of sodium chloride, 7 c.c. of a 10 per cent. solution of metaphosphate of soda and 5 c.c. of a 10 per cent. solution of bi-potassium phosphate. After these salts are added the medium must not be boiled again.

A thin layer of the medium is spread on a glass slide and allowed to cool. A drop of blood is placed on the agar film, covered and examined at 40° C. The specimen may be fixed by allowing Fleming's solution or osmic acid 2 per cent. solution to run under the cover glass. After fixation the platelets may be stained as desired.

DEMONSTRATION OF GLYCOGEN IN THE BLOOD

Two methods may be used for this purpose. The dried film; unfixed, is mounted in an iodine-gum solution.

Iodine	1 gm.
Potassium iodide	3 gm.
Distilled water	100 c.c.

Gum arabic, q.s to make a syrupy solution.

Or, the dried film is exposed to the vapour of iodine in a wide-mouthed bottle containing the crystals for fifteen minutes. It is then removed and mounted in a saturated solution of lævulose.

THE MYLIUS REACTION FOR FREE ALKALI

This reaction was introduced for the purpose of demonstrating the presence of free alkali in glass. It has been applied by Erlich as a test for alkali in blood. All slides or cover slips used must be washed in acids to remove any alkali. A 1 per cent. aqueous solution of erythrosin is acidified with acetic acid, which precipitates free iodin-eosin; the latter is filtered off and dissolved in ether or chloroform, making a yellow coloured solution, which in the presence of alkali turns red. Dried blood films are placed in the chloroform or ether solution until dark red, removed and washed in chloroform or ether, and mounted in neutral Canada balsam. The plasma stains red, the erythrocytes remain unstained, also the nuclei of the leucocytes, but the protoplasm of the leucocytes stains red, and the blood platelets intense red. The red colour indicates the presence of alkali, wherever it occurs.

EXAMINATION OF THE BLOOD BY MEANS OF CUT SECTIONS

The production of film specimens of blood in the usual way is most useful and expeditious for purposes of clinical examination, but it is not quite a satisfactory method for the study of the minute structure of the leucocytes. The technique of spreading the film and the drying of the cells prior to fixation allow of effects which are detrimental to the preservation of their delicate structures; although the general appearances, when double-stained by such stains as hæmatoxylin or methylene blue, followed by eosin, orange or acid fuchsin, or the combined fixation and staining by Jenner's or Leishman's methods are sufficiently similar to permit clinical recognition and classification. These methods provide tinctorial results upon which have been based the recognized classification of leucocytes for purposes of clinical differential diagnosis.

When one wishes to study the leucocyte from a cytological standpoint, more delicate and precise methods must be exercised in order to retain the cell-structures without distortion, and in a condition which will reveal the architecture of the living cell as accurately as possible. To this end the following technique answers admirably. Instead of making spread films of blood, it is fixed, embedded, and cut into sections.

After cleansing the finger it is pricked with a sterile glass or steel needle, and the exuding blood allowed to fall directly into the fixing solution contained in a thin glass tube about half an inch in diameter and two or three inches long. The fixative which I find gives the best results is Fleming's chromo-osmo-acetic strong solution.

Glacial acetic acid	100 c.c.
2 per cent. osmic acid solution in distilled water	400 c.c.
1 per cent. chromic acid solution in distilled water	1,500 c.c.

On sinking into the fixative, the drops of blood coagulate to a degree. Fixation is allowed to proceed for not less than two and not more than twenty-four hours, according to the proportion of blood and solution. The tube should be gently agitated during the first part of fixation, but left to stand undisturbed during the latter part, so that the finer particles and any separate leucocytes may settle to the bottom. To expedite this the contents of the tube may be centrifuged. When the supernatant liquid is quite clear, it should be decanted from the sediment by means of a fine pipette fitted with a rubber teat. Ten per cent. alcohol is then substituted for the Fleming's solution, and the same process of settling or centrifuging and decantation repeated. Ten or fifteen minutes in alcohol will suffice if the centrifuge be used, but it will take much longer, if the contents be allowed to settle in the ordinary way. By a further repetition of the process the fixed blood is passed through a series of alcohols of 20, 30, 40, 50, 60, 70, 80, 90 per cent. to absolute. The absolute will require changing two or three times to ensure complete dehydration.

In the final alcohol the centrifuge should not be used, so that loose red and white cells may settle down mixed together. After complete dehydration part of the alcohol is decanted without disturbing the sediment, and cedar wood oil is allowed to flow gently down the inside of the tube. It will fall to the bottom and the fixed blood will form a layer between it and the alcohol. Gradually the fixed blood will sink through the oil and " clear " at the same time. The alcohol is removed and the cedar oil changed as desired. Two or three changes of oil may be necessary, as it deosmiates the fixed blood; this is recognized by the oil turning brown. After clarification the tube is placed in an incubator at 40° C. until warm, the oil removed and melted paraffin (melting at 40° C.) substituted. The tube is left in the incubator until the contents have settled. Three changes of paraffin are necessary to ensure the complete removal of the cedar oil, failing which good sections cannot be obtained. When the blood has settled in the final paraffin, the tube is removed from the incubator and placed in cold water, care being taken not to shake it or allow the water to overflow into it until the upper surface of the paraffin has set, when it may be submerged. To remove the block the tube must be broken and the glass scaled off, preferably under water. The block may then be attached to the microtome carrier, orientated as desired, and sections cut in the ordinary way.

To fix a section to the microscope slide, the latter is smeared with a thin layer of Meyer's albumen, the section placed upon it and a few drops of distilled water run under the section from a pipette. The slide is then placed upon a warm porcelain slab, when the section will spread out as the paraffin softens, but the heat should not be sufficient to melt it. The water is drained off and the section gently pressed to the slide while covered with a piece of damp filter paper. The slide is left to dry, when the section will be ready for staining. For this purpose the paraffin is first removed with chloroform, then the section passed through 90, 60, 30 per cent. alcohol to water, if aqueous stains are to be used. Various forms of hæmatoxylin with acid fuchsin, orange G, or eosin give excellent results, but any suitable stain may be used. A beautiful result is obtained by using a saturated solution of basic fuchsin in 75 per cent. alcohol for several hours, and subsequently washed out, until only the nuclei retain the stain, in saturated aqueous solution of orange G. Thionin blue, followed by acid fuchsin and orange G, give an excellent triple stain. After staining the section is passed through 30, 60, 90 per cent. alcohol to absolute, cleared in clove oil or xylol, and mounted in Canada balsam. The passage through alcohols is not applicable to stains such as Jenner's and Leishman's, but these stains may be used with good results if the following procedure be adopted. After removing the paraffin with chloroform, the section is transferred to acetone and thence to pure methylic alcohol, care being taken to prevent evaporation. The above stains are used in the ordinary way, and after washing in distilled water the section is passed rapidly through two changes of acetone to xylol and mounted in Canada balsam.

By careful attention to detail with the above methods all the finer structure of the cells—nucleus, cytoplasm, archoplasm, centrosomes, granulation, and mitotic figures—may be easily examined with sufficiently high powers. The blood platelets remain separate and stain well with gentian violet or dahlia.

Marked differences will be distinguished in the appearance of the leucocytes prepared in this way, from those in which spreading and drying have been part of the process.

CHAPTER VI

MORPHOLOGY OF THE ERYTHROCYTES—VARIATIONS IN THE ERYTHROCYTES—PHYSIOLOGICAL — PATHOLOGICAL — SIZE—SHAPE—NUMBER—ISOTONICITY—DECOLOURATION AND NECROSIS—ALTERATIONS OF STAINING REACTION—POLYCHROMASIA—BASOPHILIC GRANULATION—NUCLEATION—BLOOD PLATELETS AND HÆMOCONIA.

MORPHOLOGY OF THE ERYTHROCYTES

THE erythrocytes are thin circular biconcave non-nucleated discs of a pale yellow colour, but in thick layers presenting the deeper red colour of blood. Weidenreich[1] does not consider that they are biconcave discs, but bell-shaped, and may change to a sphere or flat disc from absorption or loss of water. There is no doubt that many erythrocytes exhibit this bell shape, which reminds one of minute medusæ and this appearance I have noted many times when erythrocytes have been present in urine and when counting them in the hæmocytometer cell. In a fresh wet film sufficiently thick to allow the cells to stand on edge, the biconcavity gives them a shape similar to a dumb-bell, or in the medusa forms concavo-convex. In such a film the erythrocytes tend to cling together in the form of rouleaux, like coins leaning against one another face to face. During the process of drying, and by the action of certain reagents the erythrocytes alter in shape, shrink irregularly so that they present alternating irregular projections and depressions of the surface known as crenation, which when extreme gives them the appearance of asterisks. The size of erythrocytes varies within narrow limits. Hayem classifies them as large 8·5 μ, medium 7·5 μ and small 6·5 μ. About 75 per cent. are of medium size; the remaining 25 per cent.

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[1] *Archiv. f. mikroscop. Anat.*, vol. lxi, 1902.

equally divided into large and small. The number present in health approximates 5,000,000 per c.mm. in the male, 4,500,000 in the female, but these numbers may vary slightly in people who are quite healthy. Stained specimens show that erythrocytes are monochromatophilic, and that they have a selective affinity for acid dyes, such as eosin or orange G. While unfixed they are achromatophilic, refusing stains, but they become orange brown in the presence of iodine vapour. In fixed specimens the intensity of their staining depends upon their hæmoglobin content; they do not all stain with the same intensity; some quite faintly, others with bipolar concentration, others with marginal excess—the so-called "pessary" forms.

Although the erythrocytes have a definite shape while at rest, they are elastic and alter their outline to accommodate themselves to their surroundings, so that they become indented by each other or elongated by pressure, but return to their original shape when such influences are removed. Appearing practically homogenous when examined microscopically, their intimate structure has been a matter of much conjecture, and various views have been put forward. Weidenreich,[1] and Schäfer,[2] amongst others, believe that they are of the nature of vesicular masses composed of a limiting envelope containing fluid contents. Rollet[3] considers that they are built up of a hyaline porous stroma containing in its meshes an endosoma which holds the hæmoglobin in an amorphous condition. Overton's[4] researches led him to conclude that in all animal and vegetable cells the surface layer is impregnated with a compound of cholesterin and lecithin which acts as a protective envelope, and according to Pascucci[5] the stroma of the erythrocytes consists of about one-third of this compound. To a certain extent these views are in mutual agreement, but it is not

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[1] *Anat. Anzeiger*, xxvi, 1905.

[2] Ibid.

[3] *Pfluger's Archiv. f. Phys.*, lxxxii, 1900.

[4] *Pfluger's Archiv. f. Phys.*, 1901.

[5] *Hofmeister's Beiträge*, vii, 1905.

PLATE I.

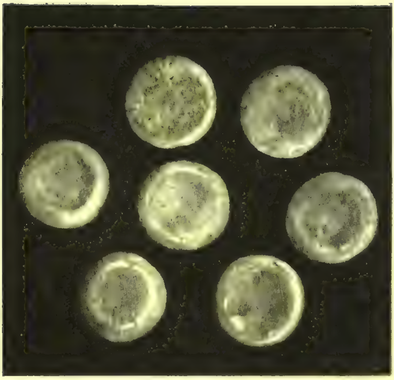

FIG. 1. R. J. M. Buchanan

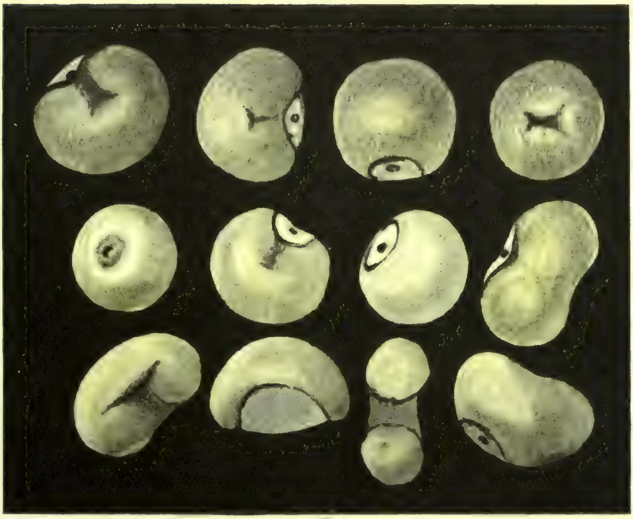

FIG. 2. R. J. M. Buchanan

easy to accept the idea that the hæmoglobin is present in simple solution in the cell, for it contains more than its amount of water can dissolve at the body temperature; and from the fact that, when fragmentation of the erythrocytes takes place, there is no corresponding escape of hæmoglobin, each fragment consisting of stroma and colouring matter, and tending, as Erlich has pointed out, to assume the biconcave form.[1] In accord with this it is a common experience to find alterations in size and shape, without alterations in structure of the cells in the plasma. Although not in solution in the erythrocyte, the hæmoglobin does not seem to be in intimate combination with the stroma for according to Rywosch,[2] trituration of erythrocytes with sand allows an escape of hæmoglobin, which colours a 0·9 per cent. salt solution when the triturate is extracted by it.

The erythrocytes take on the acid dyes in preference to basic; they may be stained with either, but, as has been pointed out before, they respond to one dye only at the same time. With the eosins they stain in shades of pink to almost a copper colour, depending partly on the depth of the stain, and the hæmoglobin content. With stains containing orange G they are coloured yellow to orange, and when more than one acid dye is present, as in Erlich's triacid solution, they select the orange rather than the acid fuchsin. This depends to some extent upon the temperature used for fixation. If not sufficiently heated the red of the acid fuchsin predominates. The latter dye is a very delicate and useful one for staining erythrocytes. In films fixed by heat or methyl alcohol the cells appear homogenous, and no stroma can be made out; but in sections of blood fixed in Fleming solution, a definite structure can be seen. The cells present the appearance of a delicate network connected at the periphery with a denser limiting layer; within the meshes of the network is granular material, which stains with the acid dye.

In sections stained with both basic and acid dyes the latter washes out the former, and the structure is less evident; but where the basic dye has not been completely cleared a definite stroma is always visible.

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[1] *Nothnagel's Dis. of Blood*, 1905, p. 51.

[2] *Zentralbl. f. Physiol.*, No. 12, 1905.

In sections stained with Heidenhain's hæmatoxylin and acid fuchsin, the structure of the erythrocytes is seen to advantage. The stroma has a greater affinity for the hæmotoxylin than the usual basic stains, and it appears in many instances as a peripheral envelope from which fine strands pass inwards to a condensed reticulated central mass, often irregular in shape, and resembling in a slight degree a nuclear body. If the hæmatoxylin be completely removed the acid stain takes its place, and the central reticulated body, if acid fuchsin be used, is stained distinctly. This reticulation with its central condensation is, in my opinion, in no way related to a nucleus or nuclear remains.

The origin and life history of the erythrocytes is a subject about which there has been much difference of opinion. It can only be considered briefly here. In the early embryo they have been looked upon as arising in two ways, one by endoglobular formation in large cells called angioblasts, which produce non-nucleated forms. This theory has not been supported by recent investigations. All observers are agreed that both in the embryo and in adult life erythrocytes are derived from nucleated cells. In the early embryo they arise from groups of mesodermal cells associated with the formation of the capillary blood vessels; further production at a slightly later period has been attributed to the liver, marrow and spleen. The principal centre of origin at birth is the bone marrow, and in the adult this is recognized as the exclusive source. In early embryonic life the nucleated erythrocyte or erythroblast is of large size, and by a series of divisions becomes smaller, forming the normoblast which remains as the nucleated erythrocyte of extra-uterine life. By loss of nucleus the erythroblast is transformed into the erythrocyte of normal blood.

The primitive cell is held by many observers to be a nucleated colourless cell, the descendants of which contain hæmoglobin. Bizzozero looks upon the marrow as an

PLATE II.

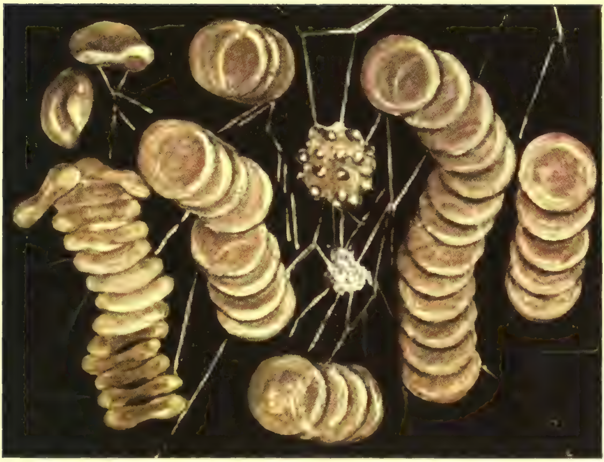

FIG. 1.

RJM Buchanan

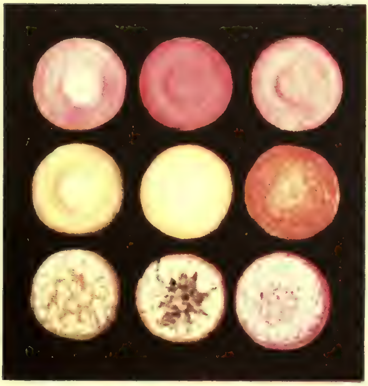

FIG. 2.

RJM Buchanan

endovascular blood gland with groups of erythroblasts within the vessels.

While some observers hold that erythrocytes and leucocytes are derived from independent colourless cells, others maintain that there is a mother cell common to both. Engel refers the origin of the nucleated erythrocytes to large embryonic cells, metrocytes, which divide into two daughter cells, each again dividing into a nucleated and non-nucleated variety, the former disappearing from the blood at the close of intra-uterine life. That the erythrocytes are derived from blood plates, as advanced by Hayem, is no longer considered tenable.

The transformation of the nucleated normoblast to the non-nucleated erythrocyte has been explained in two ways. One that the nucleus is extruded from the cell, the other that it breaks up and disappears in the cell by a process of solution, karyorrhexis and karyolysis. The first view, put forward by Rindfleisch,[1] was based upon his observations of the process in the blood of guinea-pig embryos and teased marrow in physiological salt solution. The nucleus retained a small amount of protoplasm and built up another erythrocyte. Neumann and Kölliker [2] advocate the latter view; the gradual metamorphosis can only be followed in stained specimens, but all downward graduations pointing to nuclear destruction are said to have been traced out in fœtal bone marrow, and leukæmic blood.

R. Ross, J. E. S. Moore and C. E. Walker [3] from their researches have concluded that the nucleus disintegrates, and during the process "chromo-linin" granules are to be found in the red corpuscles. They consider that this is the normal process in the evolution of the non-nucleated erythrocyte, from a comparison with similar changes which take place in the nucleated red cells of amphibia, reptiles and birds during the process of dying. They find a large proportion of such granular cells in normal bone marrow, but few in the blood stream, but in conditions of anæmia the number in the blood stream is increased. The similarity in the red cells of man to those of cells in lower animals which they describe, and which are accepted as in a dying condition, rather points to the extreme probability that such cells, when present in human blood, are in a state of degeneration and not likely to reach the fully developed condition of a normal non-nucleated erythrocyte, which is perhaps the most specialized of all cells in the body, as it has only one function to perform.

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[1] *Archiv. fur mikroskop. Anat.*, 1880, vol. xvii, p. 1.

[2] *Zeitschr. f. klin. Med.*, 1881, vol. iii, p. 411.

[3] *Transac. of the Path. Soc. of London*, vol. lviii, part i, 1907.

One must not forget that some of the so-called transition stages may be due to degenerative processes in the cytoplasm and independent of the nucleus.

Of the two opinions my personal observations lead me to believe that in the normoblast nuclear extrusion does take place, that the nucleus takes with it a small rim of protoplasm with its centrosome, that a new erythrocyte is gradually built up, and the process repeated. In sections of human bone marrow I have been able to trace various positions of the nucleus to complete separation, and the subsequent formation of a new erythrocyte. This to my mind is the more rational view compatible with a natural process, and the one to be expected rather than nuclear solution. That nuclear disintegration should take place in all normoblasts is to my mind incompatible with repeated blood formation and life. The supply would surely become exhausted early. If such a process really takes place, I can only conceive of it doing so in one out of two normoblasts, the other remaining to produce a fresh cell by division, a somewhat similar cycle to that advocated by Engel in reference to the origin of erythrocytes.

By nuclear extrusion and re-formation of erythrocytes a constant supply can be kept up, and the numerical increase demanded by growth met by mitosis of the nucleated erythrocyte as it may be required. When a special call is made, as after a hæmorrhage, mitosis will also provide for any such contingency at the time. Erlich believes that both processes take place, but in different types of cells, the transformation being by nuclear extrusion in the normoblast, by disintegration and solution in the embryonic megaloblast.

The period of life of the erythrocyte is difficult to determine. Quincke believes it to be about three or four weeks. In any specimen of blood there must exist young, adult, senile and dying erythrocytes, but it is almost hopeless to distinguish one from the other. Difference in size or resistance to external influences give no clue, but there is reason to believe that as the cell increases in age its hæmoglobin content becomes greater, a condition which may be recognized by a corresponding affinity for stains. The ultimate destruction of erythrocytes after they have lost their special function as oxygen carriers probably takes place in the spleen, and according to Warthin[1] in the hæmolymph glands, which are situated in the retroperitoneal and mediastinal tissues.

VARIATIONS IN THE ERYTHROCYTES

Variations in the erythrocytes are chiefly associated with pathological conditions, but there are certain physiological factors which influence them, principally in relation to numbers, less so to size. The average normal number has been estimated at 5,000,000 per c.mm. in the adult male and 4,500,000 in the female. In the newly-born infant the numbers may vary from four and a half to eight and a half millions, the average being about five and a half. This polycythæmia gradually diminishes from birth until about the fourteenth day, when the normal number is reached. During adult life the average number remains practically constant in health, with a tendency to diminution in old age. The state of nutrition is associated with numerical alterations, generally speaking the healthy and robust average a larger percentage than those less so, but fat people average a lower count than lean. Environment as it affects the general nutrition influences the number, and fatigue as a rule lowers it. After meals there is a gradual fall as digestion proceeds, most probably due and proportionate to the amount of fluid ingested. Fasting produces a concentration of the blood and a numerical rise, but it cannot be said to be constant or invariable. Except in so far as it is in association with digestion, exercise or fatigue, the time of day exercises no special influence.

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[1] *Journ. Boston Soc. of Med. Sci.*, 1901, vol. v, p. 414.

In women the tendency of menstruation, child-birth and lactation is to diminish the number, dependent not only on the amount of blood lost, but also on the power of regeneration. Pregnancy does not seem to produce any effect, but during the puerperium Given,[1] from an examination of twelve cases, shows the erythrocytes to have been distinctly diminished at the end of pregnancy, a further slight diminution occurring immediately after parturition (there was no case of post-partum hæmorrhage), followed by a steady increase to normal by the seventeenth day of the puerperium. As a rule the erythrocytic count depends upon the concentration of the blood, being raised by loss and decreased by ingestion of large amounts of liquids. Locally the count may be altered in the direction of gain from vaso-dilatation and loss by vaso-constriction. Hence the count may vary according to the part of the body from which the blood may have been drawn.

At high altitudes the number of erythrocytes increases, and with it the percentage of hæmoglobin and the specific gravity. This increase takes place during the ascent and diminishes during descent; above six to eight thousand feet the polycythæmia is not much greater than at that level. In people and animals living at high altitudes there is a constant polycythæmia. The specific gravity of the serum is not affected. The polycythæmia depends upon an exit of plasma from the vessels and not to an increased production by the bone marrow, for the blood picture does not reveal any condition indicative of the latter.[2]

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[1] *Journ. of Obstet. and Gynæcol. of the Br. Emp.*, April, 1906.

[2] Buckmaster, *Morphology of Normal and Pathological Blood*, p. 41.

Cold baths, massage and electricity produce a transient polycythæmia. Conditions associated with the removal of much fluid from the body cause it, such as the administration of purgatives, emetics and diaphoretics, diseases accompanied with severe diarrhœa, vomiting or profound perspirations. After severe hæmorrhage regeneration may be sufficiently active to produce a numerical increase for a time. Rapid exudation of large quantities of fluid into serous cavities, as in ascites after tapping, may result in a temporary polycythæmia. After transfusion of blood the count may remain above normal for a short time. Other conditions are the cyanosis and stasis of chronic heart disease, emphysema and asphyxia, phosphorus poisoning, acute yellow atrophy of the liver, and poisoning by carbon monoxide.

The condition known as polycythæmia vera, or cryptogenetic polycythæmia, will be described under the diseases associated with abnormal blood states.

Diminution in the erythrocytic count is indicative of and associated with all forms of anæmia, temporary or permanent. It occurs in greater or less degree, and is generally accompanied by a reduction in the amount of hæmoglobin, but the whole volume of blood need not necessarily be diminished. The lowest counts are usually found in pernicious anæmia; in chlorosis the loss may not be by any means great. Malnutrition and bad hygienic surroundings are responsible to a certain degree for oligocythæmia, but in the secondary anæmias, the principal causes are hæmorrhage, chronic exhausting diseases, e.g. malignant growths, tuberculosis, suppuration, nephritis, septic absorption, syphilis, diarrhœa, chronic diarrhœa and dysentery, and poisoning by lead, arsenic, etc. Acute febrile conditions may cause a diminution, but not necessarily; the effects of acute rheumatism and malarial fevers are well-known. Intestinal parasites, especially the bothriocephalus latus, may produce a marked oligocythæmia. As to how far the corpuscular loss may progress compatible with life and recovery, it is difficult to say, but when the count falls to 500,000 per c.mm. the result is usually fatal.

PATHOLOGICAL VARIATIONS IN THE ERYTHROCYTES

Size and shape.—In the normal blood of the adult the size and shape of the erythrocytes varies but very little. In infancy they do, and may measure from as low as 3 μ to 10 μ or more. While drying in the fresh specimen, peculiar alterations in shape occur by shrinkage of the surface, producing crenation or an asterisk appearance, but this does not take place until after a certain period. In pathological conditions changes in shape and size are notable, peculiar and generally associated with conditions of anæmia.

In the profound anæmias erythrocytes may be present which are much larger than normal, measuring from 9 μ to 20 μ or more. They are called **megalocytes** or **macrocytes,** and if present in considerable numbers constitute a blood state known as megalocythæmia. These large cells may be the result of altered osmotic conditions by which swelling is produced. Their principal origin is from large nucleated antecedent cells from the marrow. They are rich in hæmoglobin in cases of pernicious anæmia, while in the secondary forms deficiency of colouring matter is the general rule.

Erythrocytes, much smaller than normal, measuring 3 μ or less, usually globular in shape—**microcytes**—are often seen in any severe form of anæmia. Some of these, known as Eichorst's corpuscles, are deeply coloured and highly refractive. Such small cells may be due to the fragmentation of erythrocytes in the blood, or enter it as direct descendants of undersized normoblasts. When present in large numbers the condition is known as microcythæmia.

Poikilocytes are distorted and irregularly shaped erythrocytes. They must be distinguished from deformed cells due to injury or crenation. The latter condition only comes on after a period of exposure to air, while poikilocytes are present at the moment the blood is withdrawn. They may be large or small, rich or poor in colouring matter, and of the most diverse forms, resembling in outline a pear, oval, horse-shoe, spindle, etc., while some present several

PLATE III.

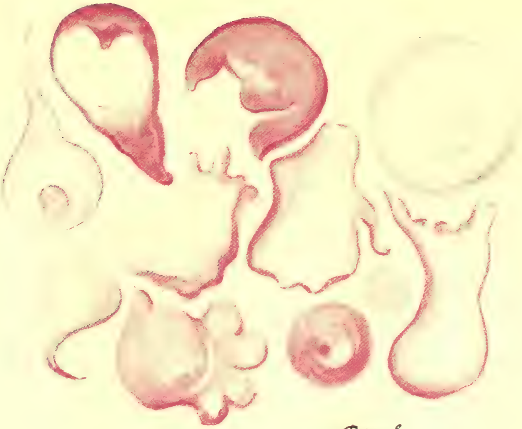

FIG. 1.

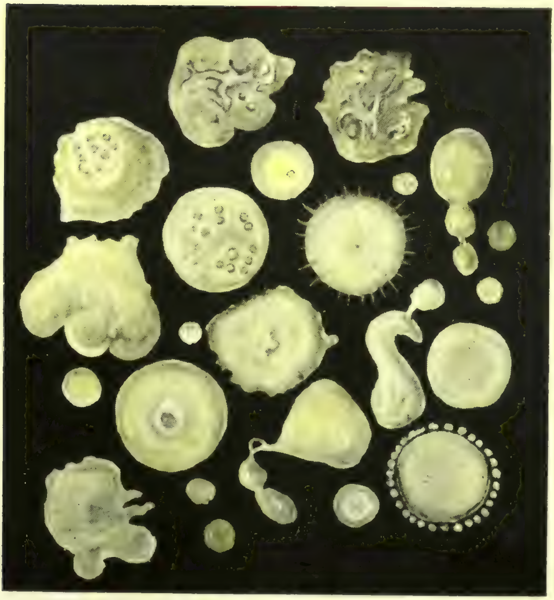

FIG. 2.

projections like pseudopodia. Very minute oscillating rod-shaped forms are sometimes seen, known as Hayem's pseudo-bacilli. The production of poikilocytes has been attributed to an amœboid activity on the part of the erythrocytes, also to their fragmentation, the fragments—"schistocytes"—retaining the normal function as oxygen carriers and acting by way of compensation to the diminished number of erythrocytes by presenting collectively a larger respiratory surface. They may also arise from defective hæmogenesis, entering the blood stream from the bone marrow.

Poikilocytes may be produced experimentally by careful heating. They are not clinically significant of any single disease, but are most numerous in cases of the grave primary anæmias, and the more severe the anæmia the larger and more irregular the poikilocytes.

Alterations in Isotonicity.—On the addition of water to blood, the erythocytes swell up, the hæmoglobin passes out into solution, and the cell eventually breaks up. If, however, an alkali such as sodium chloride be first dissolved in the water, up to a certain concentration this may be prevented. Sodium chloride of 0·44 to 0·48 per cent. does this. Solutions of substances of just sufficient concentration to prevent the escape of the hæmoglobin are said to be "isotonic." If they be more or less concentrated, they are termed "hypertonic" or "hypotonic" respectively. Even in 0·48 salt solution the erythrocytes may swell up, and to prevent this 0·6 to 0·9 per cent. solutions are required. The latter not only will prevent escape of hæmoglobin, but preserve the contour of the corpuscles.

Even in normal blood the resistance of the corpuscles is variable; they are not individually alike, some more, others less, resistant. Hence it is doubtful whether the estimation of the resistance is of any great clinical value. The escape of the hæmoglobin depends upon the sensibility of the cortical layer of the individual cell. The lower the percentage strength of the salt solution required to preserve the corpuscles, the more resistant they are, and vice versa.

For therapeutical purposes, e.g. transfusion, 0·6 to 0·9 per cent. salt solution is generally used.

The resistance of the erythrocytes is lowered in pernicious and other profound anæmias, raised in chlorosis and jaundice. Changes in other diseases have not been characteristic.

Decolouration and Necrosis of Erythrocytes.—The normal erythrocyte presents a uniform colour except at the centre, where it is paler. This uniformity is due to the even distribution of the colouring matter throughout the cell. In certain anæmias, however, such as chlorosis and secondary anæmias not usually associated with great diminution in number or colouring matter of the erythrocytes, nucleation or poikilocytosis, a decolouration of the cells, may take place. This may spread uniformly from centre to periphery, which if extreme, results in what are known as Hayem's "achromocytes" or Ponfick's "shadow corpuscles"; or it may occur in the form of irregularly distributed patches or streaks. These areas show motility within the cell, and are not to be confounded with malarial parasites. They lose their affinity for acid, and tend to respond to basic dyes such as methylene blue. Maragliano[1] and Castellino attribute this condition to endoglobular degeneration. It has also been investigated in malarial conditions by Celli and Marchiafava. Hayem described it as the "état cribriform," and Klebs as "pseudo-nucleation." A similar degeneration is the vacuolation which takes place in erythrocytes exposed to air in the wet blood film.

A more advanced stage of degeneration than the one just described is total necrosis and disintegration, which results finally in a breaking up of the cell.

Both changes may occur together or separately, but the latter condition is usually associated with the more grave pathological blood states. At the commencement of the necrotic process elevated or corrugated ridges develop in the stroma, increasing in size and number, so as to produce cell deformity. Amœboid movement follows, which with loss of resiliency transforms the cell into a poikilocyte, from which small portions project from the surface like pseudopodia, break off, and float free in the plasma. These tend to resume the biconcave form, but show an affinity for basic dyes. Decolouration and necrosis may be studied in fresh films of normal or pathological blood. In the former they are due to prolonged exposure to air, commencing in about half an hour, and reaching completion in about four hours. They occur very rapidly in pathological blood states, or may be noticed immediately the blood is withdrawn, and if so may be considered as present in the circulating blood. In pathological blood states the changes are attributed to globulicidal action of the plasma and to decreased resistance on the part of the erythrocytes rendering them more susceptible to injury even by normal plasma or external influences.

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[1] *XI Congress f. Inn. Med.*, Leipzig, 1892.

It will be convenient here to mention the peculiar change which takes place in erythrocytes, with the formation of a condensed round particle of hæmoglobin in the centre of the cell, and which stains deeply with eosin, while the rest of the cell is stained but slightly or not at all. This hæmoglobinæmic inner body described by Erlich may be extruded from the cell and remain free in the plasma, where it is supposed that it may give rise to a blood-platelet. This change is not at all an uncommon one in anæmic conditions, and as an effect of poisons such as lead, phenyl-hydrazin, nitro-benzol, arseniuretted hydrogen, etc.

Alteration of staining reaction—polychromasia—polychromatophilia.—The normal erythrocyte is monochromatophilic in that it has a selective affinity for a single acid dye, notably eosin. In blood films stained with a mixture of a basic and acid dye—e.g. methylene blue and eosin—the erythrocyte responds to the latter and not the former. From a mixture of two or more acid dyes it will select one of them to the exclusion of the others. In certain conditions this affinity is so altered that erythrocytes may stain with either acid or basic dyes, or in a mixture of these may respond to both at the same time, out of which if but one be selected it is generally the basic dye.

Erythrocytes which exhibit these peculiarities are termed polychromatophilic. The condition is most noticeable in megalocytes and their antecedent megaloblasts, in cells also which are poor in hæmoglobin or the subject of necrosis. It is also seen in the blood of newly-born animals, such as the guinea-pig, rat, or mouse. It may be present in cells as a sign of youth or senescence. Although occasionally observed in what may be considered " normal " blood, such cells must be looked upon as imperfect.

The cells may be uniformly polychromatophilic, or the change may occur in patches most commonly situate at the periphery, or in nucleated cells in the perinuclear zone. With a mixture of methyl blue and eosin the cell may exhibit a diffuse or patchy violet colour instead of the characteristic red of eosin.

The true significance of the change has given rise to much controversy. Erlich, who first described it as " anæmic degeneration," looks upon it as a degenerative process. On the other hand, Gabritschewski, Askanazy, Dunin,[1] Schaumann and others looked upon it as a sign of regeneration. Both explanations of the condition may be correct. In any case, the cell showing polychromatophilia must be looked upon as " degenerate," be it from arrested development or cytoplasmic degeneration, it implies impairment of function.

Personal observation leads me to believe that the condition is one of degeneration, and I am satisfied that the more pronounced changes are generally associated with the graver pathological blood states.

The following arguments have been put forward on behalf of the theory of cell degeneration :—

(1) That cells which exhibit the most pronounced polychromasia show other signs of degeneration, such as endoglobular changes, poikilocytosis, and fragmentation.

(2) That such changes may be produced experimentally in the blood of animals by inanition, in which formation of

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[1] Cited by Erlich and Lazarus, " Diseases of the Blood," *Nothnagel Encyclop.*

PLATE IV.

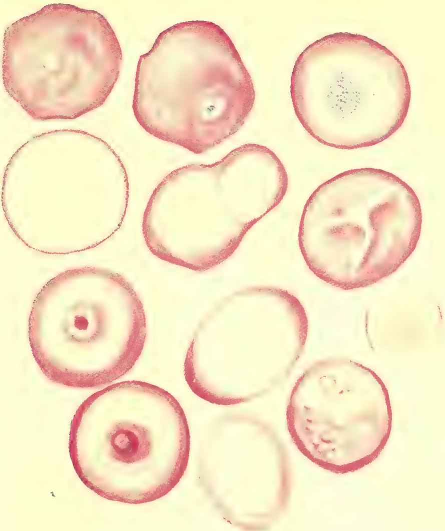

FIG. 1.

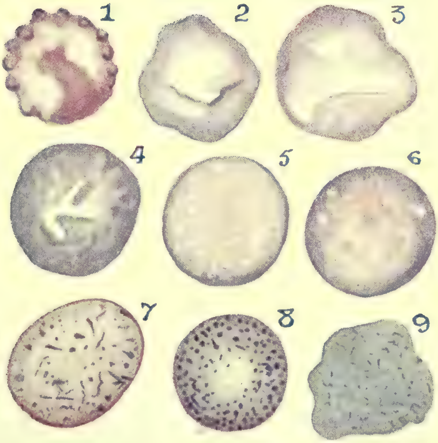

FIG. 2.

new erythrocytes in the bone marrow is practically at a standstill.

(3) If blood, the erythrocytes of which are free from polychromasia, be defibrinated and kept in a sterile tube for four or five days, and then re-examined, the change will be found in many cells.

(4) After severe hæmorrhage, polychromasia occurs prior to the appearance of nucleated erythrocytes.

(5) Although the change may be seen in both normoblasts and megaloblasts, it is rare in the former, but common in the latter.

Polychromatophilia is present in any form of anæmia, whether primary or secondary. Generally speaking, the more severe the anæmia the more pronounced the change. It is a constant phenomenon in pernicious anæmia and myelogenous leukæmia. In chlorosis, however, although the hæmoglobin content be low, it is not a marked feature unless there be a considerable diminution in the number of erythrocytes.

Basophilic granulation.—Closely allied to polychromatophilia and in some instances associated with it is the peculiar phenomenon of basophilic granulation of erythrocytes, by which they become stippled with areas reacting strongly to basic dyes. These so-called basophilic granulations may appear as dots, rods, irregular patches, and, as I have often noticed, spiral markings. These different varieties may exist separately or combined in a single cell. Erythrocytes of any shape, whether nucleated or non-nucleated, may be thus affected. The same difficulty is met with here as with polychromatophilia in the interpretation of their true nature and origin. On the one hand, they are regarded as the result of nuclear solution and disintegration, while on the other they are considered to be signs of cytoplasmic degeneration.

In favour of their nuclear origin during the process of transformation of erythroblasts to erythrocytes it has been claimed that transitional stages occur in which the granulation appears as the nucleus breaks up. Again, basophilic granulation, although said to be absent from the blood of the human embryo, is present in the erythrocytes of embryonic mice and cats, and of adult rabbits, guinea-pigs, and squirrels.

The weight of evidence, however, seems to support the opinion held by many observers that basophilic granulation is due to a specific degeneration of the cytoplasm. It may be present in blood which shows no other form of degeneration. Although it may be seen in the blood of persons who are seemingly healthy, one would expect to find it in numerous erythrocytes if it were due to nuclear disintegration, but this is not so. Nor are transitional changes between erythroblast and erythrocyte seen in normal blood. Stengel and Da Costa have failed to find such transition of nucleus to granule after careful search in the blood of pernicious anæmia or leukæmia. Grawitz and Buckmaster regard basophilic granulation as absent from the erythrocytes of normal bone marrow. I have never met with any convincing instance of transitional change productive of these granules, nor have I seen them in normal bone marrow. In a case of poisoning by arseniuretted hydrogen with marked blood destruction, which came under my observation, basophilic granulation was a pronounced feature; and although nucleated erythrocytes were numerous, I could discover no transition between nucleus and granule. In another case of suppurative panophthalmitis, associated with marked anæmia and leucocytosis, but no pyrexia, I found many erythroblasts and abundant basophilic granulation, but no connexion between the two. In specimens from a case of leukanæmia, kindly placed at my disposal by Dr. Hurter, of Liverpool, in which erythroblasts were in extreme numbers, so that with a $\frac{1}{12}$ in. o.imm. as many as nine could be observed close together in a single field of the microscope, careful observation revealed no derivation of granule from nucleus.

Erythroblasts may show basophilic granulation when their nuclei are normal, or even while undergoing mitotic division, which is strong evidence against their nuclear origin. They refuse to react to methyl-green, which is so precise a nuclear stain. In specimens stained with Leishman's stain I have always found these granules coloured a gray blue to black, unlike the ruby of chromatin.

Basophilic granulation may be produced experimentally by the action of heat or toxic substances. Grawitz [1] produced it in the blood of white mice by continuous exposure to a temperature of 37° C. to 43° C. The change appeared after about eight days, to pass off as the animals became accustomed to the heat. It was associated with ill-health, its disappearance with recovery.

Amongst toxic substances the administration of salts of copper, tin and lead, phenyl-hydrazin, nitro-benzol, methylene blue, and arseniuretted hydrogen as quoted above are known to produce the granulation. The action of lead is profound and rapid. Hamel [2] produced it experimentally with lead in animals. Pepper [3] found the granules in his own blood twenty-five hours after taking seven and a half grains of lead acetate. Moritz,[4] Hamel,[5] White and Pepper have demonstrated that this condition is a constant feature of lead poisoning and may occur prior to the cardinal symptoms or anæmia. The granules may be arranged in rings or figure-of-8 designs, as described by Cabot [6] in lead poisoning. I have noticed them so arranged and in considerable numbers in this condition. They stain red with Wright's modification of Leishman's stain. Grawitz [7] found the granules appear in the blood after the administration of preparations containing hæmoglobin, and disappear on its withdrawal. He attributes it to the absorption of toxic substances formed from the hæmoglobin in the alimentary canal.

While considering the question of their origin, one ought not to lose sight of the possibility that these basophile granules may not be all identical in nature and derivation; so that in some cases they may be composed of nuclear material, in others they may be the result of specific cytoplasmic degeneration.

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[1] *Amer. Journ. Med. Sci.*, 1900, cxx, 277. Cited by Da Costa, *Amer. Med.*, vol. v, 1903.

[2] *Deut. Arch. f. klin. Med.*, 1900, lxvii, 357.

[3] *Deut. Med. Wochenschr.*, 1901, xxvii, 68.

[4] Loc. cit.

[5] *Amer. Journ. Med. Sci.*, 1901–1902, and *Med. Rec.*, May 11, 1901; *Journ. Assoc. of Amer. Physicians*, 1901.

[6] *Med. Rec.*, March 28, 1903.

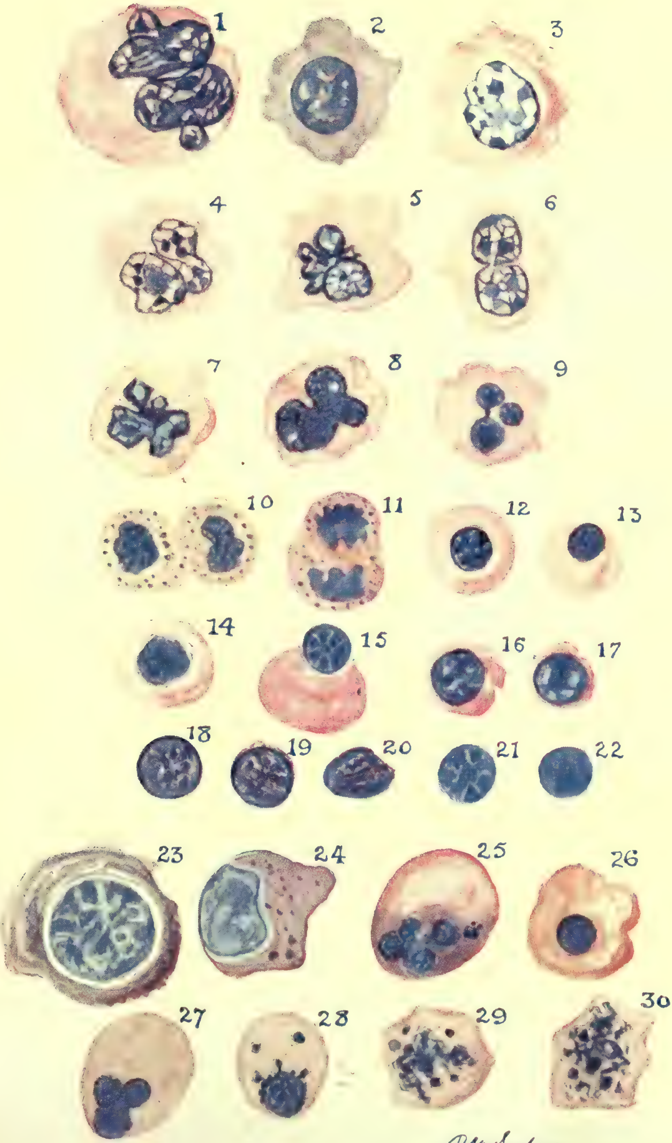
[7] Cited by J. C. Da Costa, *Amer. Med.*, vol. v, April 11, 1903.

Clinically they are present in many anæmic conditions, notably lead poisoning, pernicious anæmia, the leukæmias, Hodgkin's disease, and post-hæmorrhagic anæmia, malaria, intestinal helminthiasis, septic infections, typhoid fever, exhausting suppurations, renal disease, cirrhosis of the liver, especially when associated with hæmorrhage from the alimentary tract, malignant disease of the alimentary canal, particularly if hæmorrhagic, advanced pulmonary tuberculosis with mixed infection and hæmoptysis, ulcer of the stomach with hæmorrhage, diabetes and leucodermia. In chlorosis the change is not common, and when present may be due to intestinal auto-intoxication.

Nucleated erythrocytes.—Nucleated erythrocytes—erythroblasts—are a normal constituent of the blood of the embryo, in which they are present in large numbers. A few may remain during the first days of life, but they quickly disappear, and are never present in the blood of adults except under pathological conditions. Three varieties are usually recognized and classified as: (1) Normoblasts, (2) megaloblasts, (3) microblasts. To these another division is sometimes added which includes cells called mesoblasts, which cannot satisfactorily be placed in any of the three main classes, as they exhibit features common to more than one of them.

Normoblasts.—These are the immediate antecedents of the erythrocytes, and are present in the bone marrow. They are cells of the same size and shape as erythrocytes, with a regular outline as a rule, a cytoplasm which stains well with acid dyes rather more deeply than the erythrocytes, and possessed of a characteristic nucleus. They are sometimes polychromatophilic, and may show basophile granulation. The nucleus is distinguished by its intense affinity for chromatin stains, has a well defined outline, and a coarse meshwork of chromatin. Occupying about half

PLATE V.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the diameter of the cell, it is usually placed excentrically, may even project from it as if being extruded, and free nuclei may be seen in the plasma.

Variations in the shape of the nucleus are common, so that in some cells it may appear double, treble or multipartite ; each portion being attached to another by a delicate thread. When so divided the individual portions stain, as a rule, more densely than usual. Mitosis is occasionally observed, and two nuclei are not uncommonly present in a single cell. In stained specimens nuclei may exhibit the appearances of amitotic division.

Megaloblasts.—These cells are much larger than the preceding variety, often several times the size of the normal erythrocyte and measuring from 10 μ to 20 μ in diameter. They are present in the fœtal bone marrow, but are said not to be a normal constituent of the marrow in the adult. With the latter view I do not concur, having noted nucleated erythroblasts of such size and appearance in sections of adult human bone marrow that they were indistinguishable from megaloblasts.

The cytoplasm is often polychromatophilic, may exhibit areas of decolouration or basophile granulation, and have an irregular outline—poikiloblasts. The nucleus is larger than that of the normoblast, may be central or excentric in the cell, has a poorly defined outline, the chromatin staining feebly, and the meshwork fine. Between the nuclear margin and the cytoplasm there is usually a clear hyaline-looking rim. This is more often present in cells fixed by heat, and stained by Erlich's triple stain. It is not constant by any means in cells prepared in other ways, even when dried prior to fixation ; I have never seen it in cells fixed by Fleming's solution.

In megaloblasts which tend to poikilocytosis—poikiloblasts—the alteration in shape is often associated with convolution and irregular distortion of the nucleus. In some cases this may proceed to definite fragmentation. Thus there seems to run a parallel between the fragmentation of the poikilocyte which has been previously mentioned and the fragmentation of the megaloblast, so that as in the one, microcytes, so in the other microblasts may be produced. Further, this association of poikilocytic change with nuclear alteration may account for some instances in which such basophilic granulation is regarded as due to nuclear disintegration.

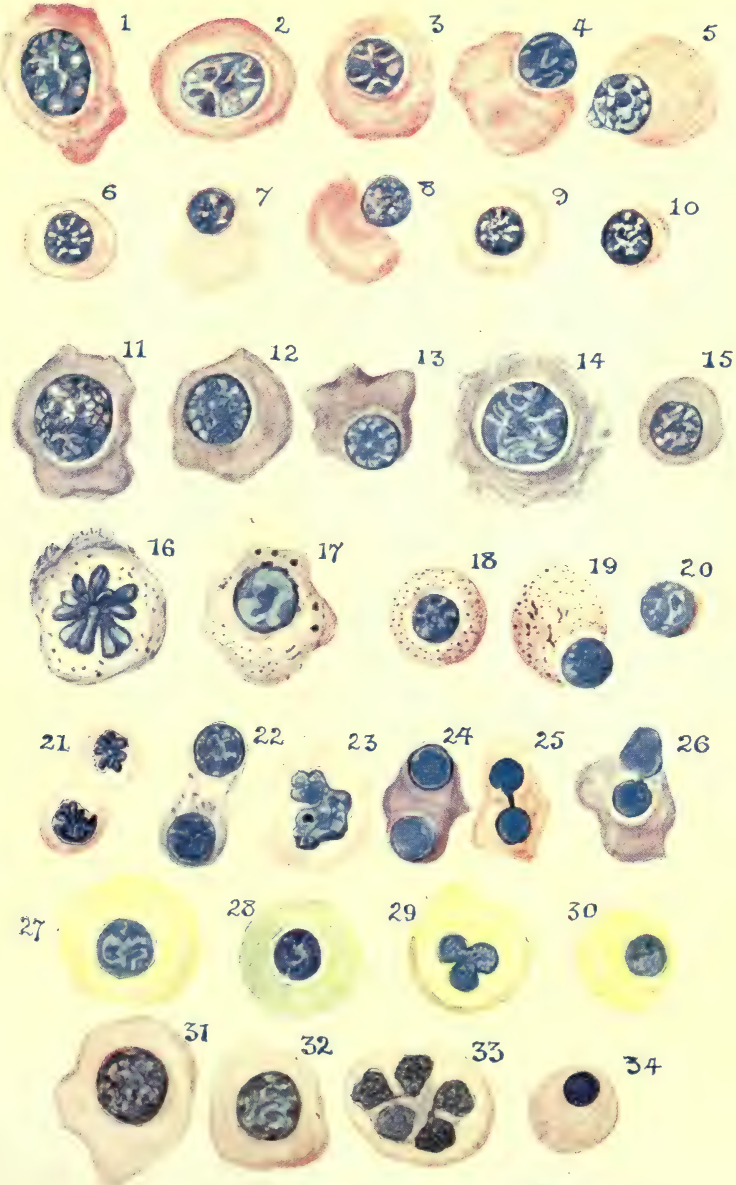
Microblasts.—These are rare forms of erythroblasts. Much less in size than normoblasts, they possess a small deeply-staining nucleus and a narrow, very often irregular, rim of cytoplasm. Their clinical significance is doubtful. The author considers that they stand in the same relation to megaloblasts as microcytes to poikilocytosis.

Atypical forms.—Although the three types described above are characteristic and easily recognized, examples are not uncommon of cells which do not conform exactly in type to any one of them, and great difficulty arises in their classification, in that they may present features common to both normoblast and megaloblast.

Endeavours have been made to allocate such cells by peculiarities of shape, size, or appearance of nucleus, but the results are often disappointing and uncertain. Limitations of size are but arbitrary. Nuclear architecture and staining reaction offer a more promising basis for distinction, but one cannot accept these as absolute ; and the fact remains that one meets with erythroblasts the true nature of which it is impossible to determine by measurement, staining reaction or peculiarities of nuclear network.

Clinical significance.—Clinically, the appearance of normoblasts in the blood stream is a departure from the normal ; for although they exist in the marrow as antecedents of erythrocytes, they do not appear in the blood except under conditions which are associated with special calls upon the bone marrow to complement erythrocytic loss. They are found in varying numbers after hæmorrhage, in severe secondary and primary anæmias. Occasionally they occur suddenly and in profusion in post hæmorrhagic anæmia, chlorosis, primary pernicious anæmia and leukæmia. This rapid overflow into the blood stream, termed a " blood crisis " by von Noorden, does not last long, but is followed by a decided increase in the erythrocytic count. At any

PLATE VI.



RJMBuchanan

time their appearance is regarded as an expression of blood regeneration.

Megalobasts occur less commonly and in fewer numbers than normoblasts. Their presence is indicative of a reversion, in lesser or greater degree, on the part of the marrow to the embryonic type, as they are normal elements of the marrow in the early fœtus. They give rise to megalocytes in the blood, and if the latter are found in large numbers, although megaloblasts be absent, it points to the presence of the latter in the bone marrow. Never found in normal adult blood, they appear as a characteristic feature in primary pernicious anæmia, Bothriocephalus anæmia, nitrobenzol poisoning, less so in leukæmia, lead poisoning and other forms of extreme anæmia. They are to be found in the anæmias of early infancy and childhood. Megaloblastic blood crises are said not to occur. Megaloblasts and normoblasts may occur together, and in certain conditions in which the former predominate their disappearance may be coincident with or followed by an increase in the latter. When both forms are present, degeneration and regeneration are regarded as going on at the same time.

The fate of the nucleus in both forms of erythroblast has already been noted, but the question arises as to the relation, if any, between megaloblast and normoblast. Can the former be transformed into the latter ? The matter is still one of controversy. On clinical evidence it has been upheld that so different are the blood pictures of essential pernicious and secondary anæmias that clinically two independent groups arise—one, megaloblastic, the other normoblastic—and that there is no relation between the two. On the other hand, a definite relation between the two forms of erythroblasts has been upheld on developmental grounds. In the embryo megaloblasts which are present early are followed by normoblasts. In infancy and childhood the morphology of the blood is less constant than in the adult, and more easily altered by conditions which influence the bone marrow ; blood genesis is more easily disturbed, so that in slight anæmias the picture may resemble that of a severe adult anæmia with the presence of megaloblasts and normoblasts. In a case of nitro-benzol poisoning Erlich and Lindenthal observed the presence of numerous erythroblasts; at first normoblasts outnumbered the megaloblasts, but later on the latter predominated.

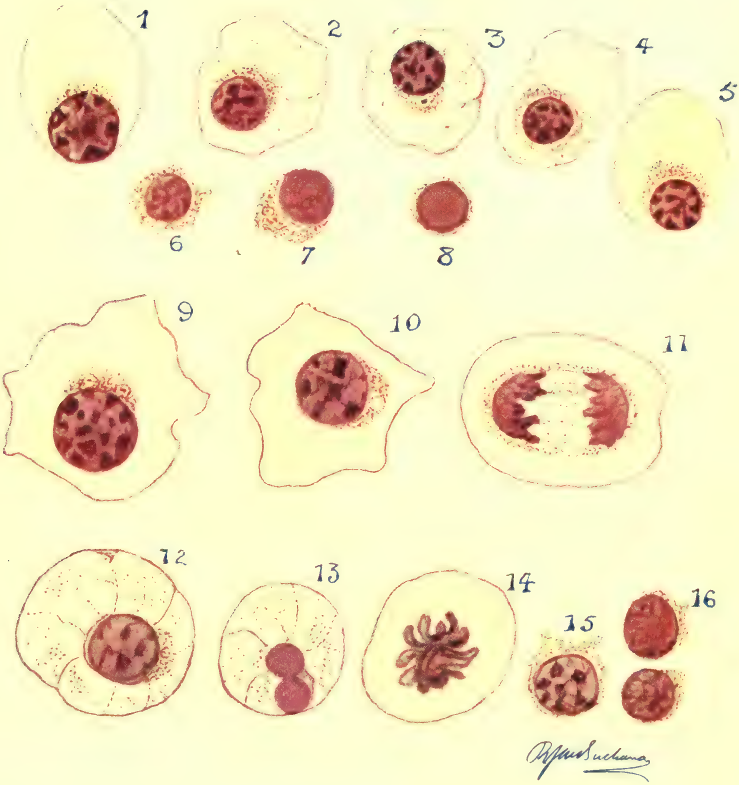
In the adult both forms may occur together, and if megaloblasts are most numerous their disappearance is not seldom coincident with or followed by a normoblastic increase. In Bothriocephalus anæmias the blood picture is not always constant. In some cases it may be a simple anæmia, in others normoblasts may be present, in others megaloblasts, or both together. Recovery is generally associated in the megaloblastic or composite forms by a passage from the former through a normoblastic stage, the latter disappearing as the blood returns to normal. There are many examples of cells of an intermediate character, the true nature of which is so difficult to determine and which allow of different interpretation, which crop up in severe anæmias, that it is almost impossible to classify them accurately. May not some, at least, of these be transitional forms? My own observations lead me to the conclusion that the normoblast is directly derived from the megaloblast even in the adult, and that megaloblasts are present in adult marrow. In sections of human bone marrow I have noted many variations in size of the nucleated red cells, some of which could only be regarded as megaloblasts.

BLOOD PLATELETS

When a drop of fresh human normal blood is examined with the microscope, small bodies of varied shape and appearance are seen to arise in the plasma. These are the blood platelets. About 3 μ in their longest diameter, their shape may be round, oval, biconcave or flat, spindle, diamond-shaped and tailed, or irregular with several processes. They are homogenous or granular, with an ill-defined edge, and show a tendency to clump together in refractile clusters from which strands of fibrin radiate.

By using solutions such as have been suggested for counting the platelets, they do not clump to the same extent and

PLATE VII.



may be examined singly. For the study of these bodies the method introduced by Deetjen [1] is perhaps the most satisfactory. He uses a preparation made by dissolving 5 grammes of agar in 500 c.c. of distilled water and boiling for half an hour. After filtering while hot, to each 100 c.c., 0·6 gramme of sodium chloride and 8 c.c. of a 10 per cent. solution of metaphosphate of soda are added; finally 5 c.c. of a 10 per cent. solution of bipotassium phosphate. A thin layer of this agar is spread on a glass slide, a drop of blood placed on it, and covered. Large numbers of platelets, irregular in shape with many processes, and a so-called nuclear centre are seen. They are said to show slight amœboid movement sufficient to produce change of shape, not of place. The idea that platelets disappear rapidly on exposure to air is erroneous; they are not easily destroyed, and resist the action of agents which hæmolyse the erythrocytes.

The platelets are alkaline in reaction and give an intense red stain with iodine-eosin, also the reaction for glycogen. Some contain hæmoglobin, others not. As to their origin, these bodies first discovered by F. Arnold [2] in 1845, and subsehuently described by Schultze,[3] Hayem [4] and Bizzozero [5] were for a long time regarded as a third morphological element in human blood and existing preformed in the plasma.

From 180,000 to over 600,000 have been noted in a cubic millimetre of blood. In fixed specimens their structure can be demonstrated by many basic stains. Leishman's stain is useful. In sections of blood fixed in Fleming's solution they remain separate, and can be easily examined. As a rule, their edges are ill-defined and stain faintly; the central portion is granular and stains like chromatin. Many of the oval or wedge-shaped platelets show a clear vesicular spot in the centre or towards the thick end.

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[1] *Virchow's Archiv.*, vol. clxiv, p. 239, 1901.

[2] *Handbuch der Anat. des Menschen*, 1845.

[3] *Arch. f. mikros. Anat.*, i, 1865.

[4] *Arch. f. Physiol.*, vol. v, 1878.

[5] *Virch. Archiv.*, xc, 1882.

Much doubt has recently been cast upon the original idea that they are preformed elements of normal blood, which by some observers were held to have arisen by extrusion from the red corpuscles, or even by budding of leucocytes. All the more recent evidence points to the conclusion that they do not exist in normal blood, but are artefacts produced during the process of examination. In pathological conditions, however, they do occur in the blood, and many may arise by extrusion from the erythrocytes. In severe anæmias and by the action of poisons which produce destruction of the erythrocytes, these bodies are present. By careful manipulation of normal blood and under conditions which do not tend to produce alterations in the plasma, preparations can be made which show no platelets, but by the action of the so-called preservative fluids upon the same plasma many platelets are seen to form rapidly. Although easily produced from plasma, they cannot be obtained from serum. That they may be derived from both plasma and erythrocytes can be demonstrated by the action of a 1 per cent. solution of potassium oxalate. The bodies which appear first are derived from the plasma; later other bodies are seen to be extruded from the erythrocytes and contain hæmoglobin.

The significance of the platelets is not fully known. That they are intimately related to the process of coagulation is established. ~~Blood freed from platelets will not coagulate.~~ That there is any definite relation between the number of platelets and the rate of coagulation is not certain. Although these bodies are now regarded as absent from normal blood as preformed elements, they do occur in pathological conditions, but their clinical significance has yet to be decided. They are said to be increased in chlorosis, post hæmorrhagic and pernicious anæmia, leukæmia, pneumonia, rheumatoid arthritis, tuberculosis and plague; but reduced in hæmophilia, purpura and acute fevers.

HÆMOCONIA OR BLOOD-DUST

Scattered throughout the plasma are innumerable minute colourless, refractile particles, spheroidal or hour-glass-shaped, which were termed hæmoconia or blood-dust by Müller. They are in constant Brownian motion, but not amæboid. They resemble micrococci, fat droplets, or granules of leucocytes. They are not fat, and are not connected with the formation of fibrin. They have been regarded as free leucocyte granules, and endowed with protective properties associated with immunity. Some may be derived from disintegration of the cellular elements. They are admirably portrayed by dark ground illumination, and for this purpose a Reichert's condenser for the illumination of fine particles is most useful.

Their clinical significance has not been determined.

CHAPTER VII

MORPHOLOGY OF THE LEUCOCYTES—CLASSIFICATION—LEUCOCYTES OF NORMAL BLOOD—ADDITIONAL VARIETIES OCCURRING IN ABNORMAL BLOOD STATES—CELL GRANULATION—AMŒBOID MOVEMENT AND PHAGOCYTOSIS—IODOPHILIA—SUDANOPHILIA.

THE LEUCOCYTES

THE position of the leucocytes and their relation to disease is one of the most interesting sections of hæmatology. First discovered in the blood by Hewson in the year 1773, their recognition in pathology was made by Virchow in 1845 in his discovery of leukæmia. Wharton Jones, in 1846, recognized and described the leucocytes as "granular" and "nucleated" cells, and divided the former into fine and coarse varieties. This distinction was confirmed by Rindfleisch in 1863, but not until 1865 was there much additional knowledge gained until Max Schultze further investigated the histology of the blood and perfected it as far as was then possible. He classified leucocytes as (*a*) a small cell with a large nucleus and small amount of protoplasm; (*b*) cells similar, but with more protoplasm and possessed of slow amœboid movement; (*c*) cells with fine granules, with one or more nuclei also amœboid, the typical white blood cell, very numerous; (*d*) cells with coarse granules also amœboid. This classification was adopted until Erlich in 1878, by his researches, threw new light on the subject. He based his researches on the powers of staining by various anilin dyes. He confined his attention particularly to the granules of the corpuscles, and classified them according to their size and elective affinity for basic, acid, or neutral dyes. With the aid of these dyes, Erlich was able to differentiate certain classes of granules in cells as follows : (α) **Eosinophile,** or oxyphile, staining with acid dyes ; (β) **amphophile,** staining with an acid or basic dye ; (γ) **basophile,** staining only with basic dyes, and the granules large ; (δ) **basophile,** and granules small ; (ϵ) **neutrophile,** staining only with a "neutral" dye. In human blood, according to the presence of specific granules in the leucocytes, he recognized four varieties of cells : (1) Small cells, no granules, the lymphocytes ; (2) finely granular cells with neutrophile reaction in man, and a polymorphous nucleus, the "neutrophile" cells (this cell being amophophile in rabbits and guinea-pigs) ; (3) coarsely granular cells with oxyphile reaction, the eosinophile cells ; (4) finely granular cells with basophile reaction. Kanthack and Hardy[1] subsequently discussed the results of Erlich and his classification, and held that no neutral dye can be formed by the mixture of an acid and basic dye, and that the so-called "neutrophile" cell of Erlich does not exist as such ; but that it is in reality an oxyphile granule that Erlich considers neutrophile. This enables us to adopt a simpler classification into "oxyphile" and "basophile." To these must be added the non-granular or "hyaline" series. In their early stages all leucocytes are non-granular or only slightly so, and it is during their further development to their adult condition that granulation becomes complete, but only in certain varieties ; so that there remains a non-granular class to which the term "hyaline" " has been applied.

The recognition of the two main classes—granular and non-granular—can be made with ease in the fresh specimen, but modifications in the illumination of the microscope field are necessary, and for this purpose no better method than a dark ground illumination with reflected light such as is afforded by the use of Reichert's new reflecting condenser for rendering visible ultra-microscopic particles.

In clinical hæmatology, to study the leucocytes and differentiate them properly, one must of necessity resort to fixed and stained preparations or blood films. It is by the examination of normal and pathological blood states by approved methods, taking into consideration both reaction to dyes and their general or nuclear architecture, that a classification has been arrived at and generally accepted. A truer basis for classification would be afforded by a complete knowledge of their origin and development, but up to the present this has not been obtained, and seems beyond the limits of experimental research.

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[1] *Journ. Physiol.*, London, 1894.

The structure of a leucocyte conforms to that of cells generally. Taking, for example, the ordinary polymorphonuclear cell, one recognizes the irregularly formed nucleus and a fine reticulated and granular cytoplasm with the appearance of a network with more deeply staining portions at the junctions of the threads. In cells which have been fixed with Fleming's strong solution the reticulum appears condensed at a certain point close to the nucleus, and forms a structure known as the archoplasm, in the centre of which lies the centrosome. To clearly define this body, one requires a magnification of 1,500 to 2,000 diameters. These finer structures are rarely visible in leucocytes as seen in the ordinary dried and stained blood film, unless much care has been exercised in the spreading. (I have specimens showing them in blood films made in the older method by means of spreading between two cover slips.)

CLASSIFICATION OF LEUCOCYTES IN NORMAL BLOOD

NON-GRANULAR : MONONUCLEAR—

- **Lymphocytes :**—(*a*) small, (*b*) large ; nucleus round, 20–30 per cent.
- **Large mononuclears** (hyaline) **:** Nucleus oval, 1–2 per cent.
- **Transitionals :** Nucleus indented (may contain a few neutrophile granulations), 2–4 per cent.

GRANULAR : With lobed or polymorphous nucleus—

Neutrophiles : Polymorphonuclear, fine neutrophile (oxyphile) granules, 70–90 per cent.

Eosinophiles : Bi-lobed or polymorphous nucleus, coarse eosinophile granules, 0·5–4 per cent.

Basophiles : Tri-lobed nucleus, fine basophile granules, 0·5 per cent.

ADDITIONAL VARIETIES OCCURRING IN ABNORMAL BLOOD STATES

Myelocytes :
- Non-granular.
- Neutrophile.
- Eosinophile.
- Basophile.
- Mixed granulation.

Myeloplaxes :

Small neutrophile pseudo-lymphocytes.

Turk's "irritation forms." Reizungsformen. Mononuclear, non-granular cells.

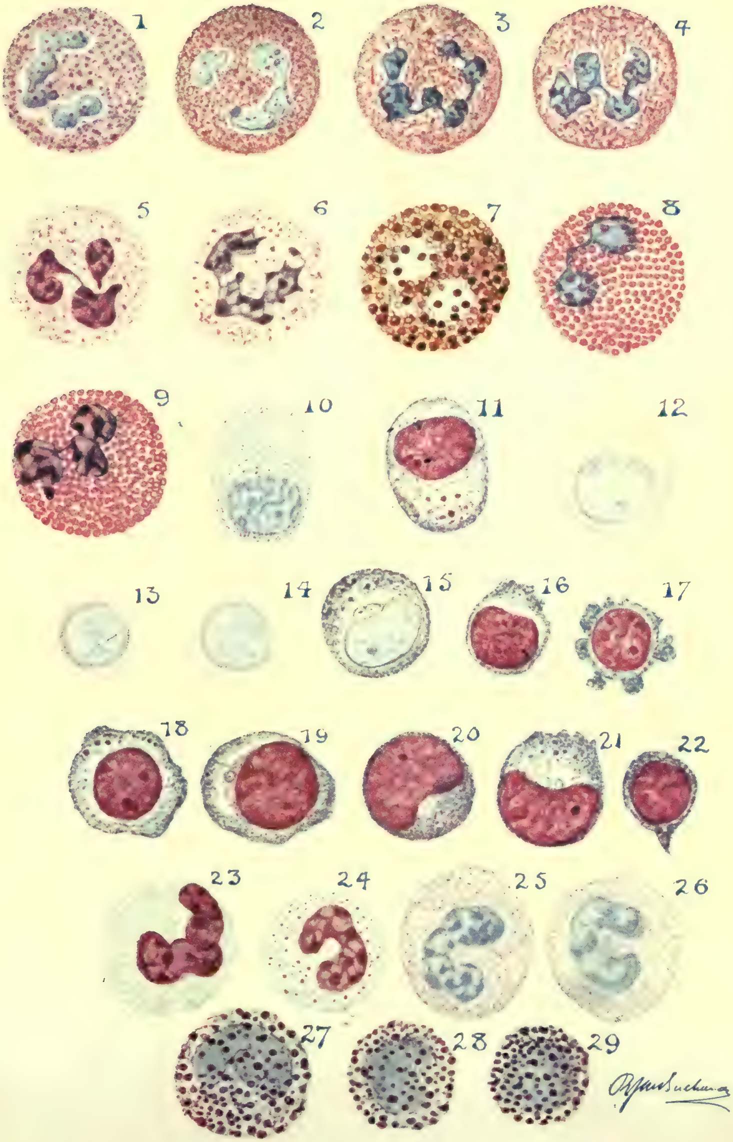
Leucoblastic cells.

LEUCOCYTES OF NORMAL BLOOD

Lymphocytes.—These are small cells, about the size of an erythrocyte, although the larger variety may be twice that size. In normal blood, if spread in a fairly thick film, but few of the large sized cells are seen, but in thin films they vary much, and as a rule the thinner the film the larger they appear, so that it would seem that the larger varieties are due to pressure in spreading. The cell contains a relatively large round nucleus surrounded by a narrow rim of cytoplasm, which may be more abundant at one pole, or appear as if drawn out, so giving the cell a pear-like shape. In such cells the nucleus appears excentric in position. Usually round, the nucleus may be slightly indented at one side, or there may even be two. It generally contains two nucleoli situated widely apart.

Stained with Erlich's triacid stain the lymphocyte is recognized by the nucleus, which stains in various shades of blue; the surrounding cytoplasm may be almost invisible, and stains at the most a faint pink and showing no granulation. With strongly acting basic dyes, or a mixture of a basic and acid dye, such as methylene blue and eosin, the cell exhibits an affinity for the basic dye, both nucleus and cytoplasm taking the stain. In occasional instances I have noted that the cytoplasm may respond to the eosin. No stain is so useful for these cells as Leishman's which gives the nucleus a rich ruby colour, and the surrounding cytoplasm a dull blue. With this stain it can be seen quite easily that the cytoplasm is reticulated, and not strictly homogenous, but containing fine but deeply staining basophile granules. The peripheral zone stains most deeply, fading off as the nucleus is approached. In the bay of an indented nucleus is a clear space in which the centrosome is situated. From this clear area a lighter band encircles the nucleus separating it from the denser protoplasm. In some cells the centrosome is surrounded by a finely granular, pink stained archoplasm. In the cytoplasm of many cells with Leishman's stain, several fairly large "ruby" granules may be seen, exactly like the nucleus in colour, which seem as if derived from the archoplasm. Although classed as a non-granular cell, there is much in its appearance to throw doubt upon this view, and I consider that, although not so characteristic and definite as the granulation of other leucocytes, it certainly is present in these cells. The cytoplasm often shows an irregular margin with frayed edges, more on one side than another, but in some cells it may be all round. In others, portions of it may protrude and appear as buds, occasionally separated from the main body by a clear space. These particles are, as a rule, more intensely basophilic than the rest of the cytoplasm from which they have become detached. In a perfect film the lymphocytes show a dual structure of the cytoplasm. (*a*) A basophile reticulum with honeycomb-like apertures. (*b*) Enclosed in this reticulum is a transparent gelatinous substance in

PLATE VIII.



close association with the archoplasm, and which like it takes on a faint pink with eosin. This material passes out in long, fine prolongations through the apertures of the basophile reticulum, and which give to the cell a starred or radiate appearance. These flagellate prolongations must not be confused with the basophile fringes and buds. The cytoplasm gives a marked alkaline reaction with iodin-eosin; it does not contain iodophilic granules.

Large mononuclear (hyaline) cells.—These cells are much larger than lymphocytes. They are two or three times the size of erythrocytes. The nucleus is large and oval, staining feebly with basic dyes, containing a nucleolus, and situated excentrically in a relatively large amount of cytoplasm which is also feebly basophile. The cytoplasm has the appearance of ground glass, and is less basophilic than the nucleus; it contains no granules, but the opacity which it exhibits is due to innumerable fine amorphous points in it which take the stain.

With Leishman's stain the nucleus takes on the ruby colour, and the cytoplasm appears more reticulated and possessed of a few ruby granules, so resembling the lymphocyte, but staining less deeply. With the triacid stain the result is similar to the lymphocyte.

Transitional cells.—These cells are about the size of the large mononuclears. The nucleus shows varieties of transition between the indented mononuclear and the irregular polymorphonuclear cell. As a rule, it is deeply indented, crescentic in form, or even twisted, but not possessed of that multipartition so characteristic of the "polymorph." The nucleus stains fairly deeply, and shows a reticulum with more deeply stained chromatin masses. The cytoplasm has an eosinophile tendency to stains containing this dye, and with the triacid mixture exhibits a slight neutrophilic granulation. It is a difficult thing to decide their position as transitional cells. In normal blood they are rare. Whether they are a transition between the large mononuclear and the "polymorph," or between the neutrophile myelocyte and the latter, is uncertain. All varieties may be seen, from those in which the nuclear alteration is so slight as to link them with the large mononuclears or neutrophile myelocytes, on the one hand; or so pronounced as to render it difficult to differentiate them from the "polymorphs," on the other. Though not common in normal blood, they may become a marked feature of the microscopic field in myelogenous leukæmia. Personally, I am inclined to regard the transitional cell as a stage between the non-granular or neutrophile myelocyte and the "polymorph." Clinically, it has been found convenient to classify these cells with the lymphocyte and large mononuclear groups, and until a definite clinical significance can be established for them as an individual group, such a classification may stand. At the same time, where marked alteration in the nucleus has taken place, there is just as much reason to claim them and classify them with the "polymorphs."

Neutrophiles.—Polymorphonuclear leucocytes ("polymorphs"). These cells measure from $10\,\mu$ to $12\,\mu$ in diameter, and are the most conspicuous leucocytes of normal blood. The distinguishing feature of the cell is its markedly chromatic and irregular nucleus, which may be of almost any shape, with many lobes joined one to the other by fine bands of chromatin. The lobes or chromatin masses stain deeply with the usual nuclear stains, and this intense staining affinity surpasses that of any other leucocyte. In shape they vary greatly, resembling alphabetical letters e.g. E, V, S, U, Z, in some the nucleus may be "wreathed," and, as occurs during active amœboid movement, form "rosettes." Although so irregular during activity, the nucleus tends to resume a spherical shape if the cell be allowed to die slowly. The chromatin forms dense masses at the internodes of a nuclear network. No nucleolus can be distinguished. With Jenner's or the triacid stain, the nucleus is not so deeply coloured as when methylene blue, hæmatoxylin alone or Leishman's stain are used. The cytoplasm is built up of a fine reticulum, the microsomes of which appear as a dense congregation of granules. These granules are irregular in shape, and vary in their affinity for stains; by any method some cells show none, or but few, while others are crowded. With the triacid stain they take on a violet colour in a well prepared specimen; but according to the degree of heat used, and the length of time they are subjected to it, they may appear pink, orange-pink, red, or reddish-violet in proportion to their reaction to the acid fuchsin or orange G.

Strictly speaking, these granules are oxyphile and stain with acid dyes such as eosin, acid fuchsin, or orange G, and they are definitely eosinophile when Jenner's or Leishman's stain is used, especially the former. As a rule, they do not stain if methylene blue be used alone, but it is held that the granules may appear oxyphile, basophile or neutrophile according to the solvents of the dyes and methods of fixation. The granules are more densely packed towards the periphery of the cytoplasm, less so near the nucleus, and in cells fixed by heat a clear non-granular zone may be seen surrounding the nucleus. In film specimens the centrosome cannot be identified in the general cytoplasm. In cut sections of perfectly fixed blood it is plainly visible in the archoplasm. Taking all things into consideration, it is better to use the term "neutrophile" for these cells when speaking of the granulation, or "polymorph" when distinguishing them from others by the nucleus.

The cytoplasm is alkaline in reaction, and under certain circumstances possesses iodophilous particles.

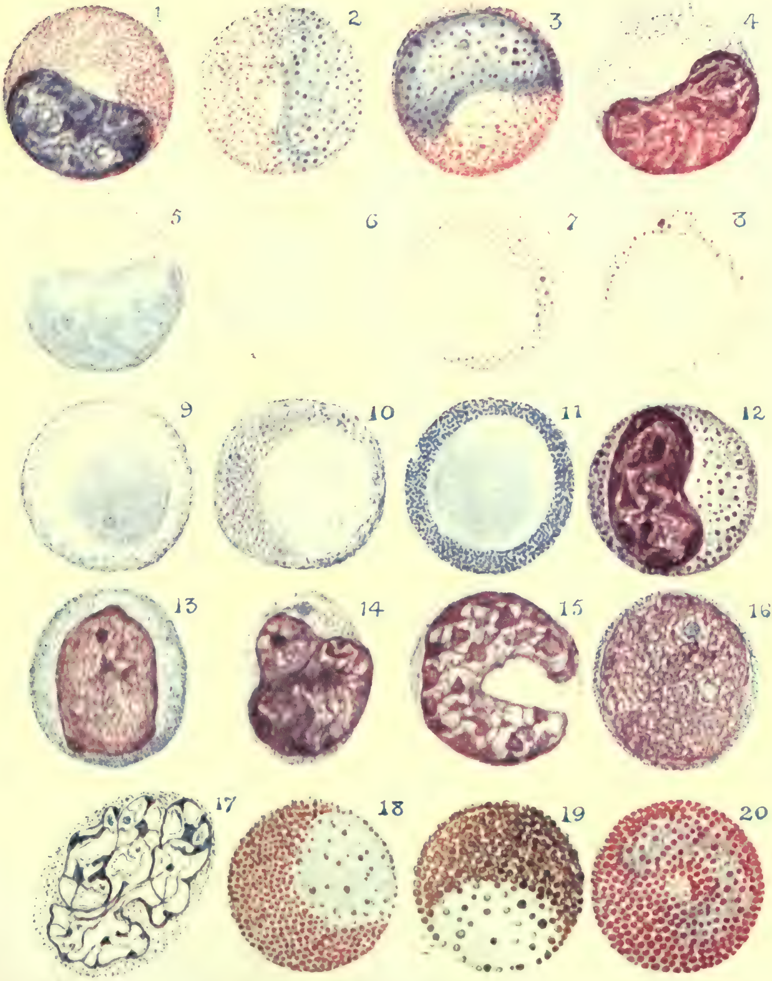
Eosinophiles.—These cells are rather larger than the neutrophiles, and are so called from the intense affinity of their granules for acid stains, notably eosin. They are thus distinguished from all the other leucocytes of normal blood. The granules are large, spherical, and highly refractile. The periphery of the granule appears to stain more deeply than the centre. Not all the same size, they may be equally distributed over the cell, or radiating from the archoplasm in parallel lines. They may be so closely packed as to obliterate portions of the nucleus. They show a marked attraction for the eosin in any stain containing it; with the triacid stain they vary in colour from an orange to a deep purple brown in the same cell.

Unless due care be taken in the preparation of the film, many cells appear ruptured, the nucleus having a few granules attached to it, while the majority are scattered around, but with a marked tendency to form regular radiations from a centre. The general protoplasm of the cell stains feebly or not at all, but I have seen cells from which the granules have been removed showing a feebly basophile reticulum with spherical meshes.

The nucleus, as a rule, has not such a marked chromatic staining affinity as the neutrophile leucocyte. It may be polymorphous or bi-lobed, with the two masses at either end of a narrow strand, crescentic in shape and with the concavity towards the centre of the cell. With the triacid stain it is feebly coloured, but well defined by methylene blue, hæmatoxylin, Jenner's or Leishman's stain.

Basophiles.—These cells are rare in normal blood, and do not exceed 0·5 per cent. of the leucocytes. They are most numerous after meals. Not quite as large as the neutrophile cells, they possess an irregular-shaped nucleus, generally tri-lobed, which does not stain deeply, being poor in chromatin. The granules vary much in size and shape. In some cells they are numerous and small, in others larger and few. They react strongly to basic dyes, but with methylene blue often react metachromatically in such a way that the granule appears purple or even deep crimson. As they are not refractile like the coarse eosinophile granule, they cannot be seen in the fresh specimen. Soluble in water, they are not so easily stained with dyes having water as a solvent; but by Jenner's or Leishman's method they are very evident. Acid dyes do not reveal them, so that they are not visible in specimens stained by the triacid mixture. With methylene blue followed by iodine solution they are rendered deep brown or even black. The granules thus overshadow the nucleus in depth of colour.

PLATE IX.



ADDITIONAL VARIETIES OCCURRING IN ABNORMAL BLOOD STATES

Myelocytes.—Non-granular. These are large cells not differing very much from the variety next to be described, except that they are free from granules. They are mononucleated cells, and may be regarded as the predecessors of the granular varieties, by the development in them of neutrophile, eosinophile, or basophile granulation. Between the non-granular and those containing specific granules, all stages of transition may be found.

Neutrophile myelocytes.—These are large cells, three or four times larger than the erythrocyte when mature, but quite small varieties occur. The nucleus is spherical or reniform in shape. In sections of fixed blood the spherical nucleus is quite common, but in films the reniform is more often seen. It is generally situated to one side of the cell, with the concavity inwards. It stains fairly well with basic dyes, but best of all with hæmatoxylin. With the triacid stain it appears pale blue and indistinct. With Leishman's stain it is coloured a ruby purple and shows a close network with several masses of chromatin. At each pole of the nucleus is a nucleolus more faintly stained, but denser at the periphery. Some nuclei are more twisted than others, and appear horse-shoe shaped, a stage of transition to the polymorphonuclear varieties. Occasionally cells with two nuclei occur, generally at opposite poles of the cell. These large myelocytes possess a considerable amount of cytoplasm, and a definite archoplasm surrounding a single or double centrosome. Derived from the archoplasm, and as internodes of a delicate reticulum, the granulation of the mature cell is well-marked. The granules are small or medium in size, similar to the "polymorph," more densely packed at the periphery of the cell, leaving a clearer space in the bay of the indented nucleus. They are neutrophile in staining reaction, and with the triacid mixture are rendered very evident, varying in colour from pink to darkish violet, and in many cells they are so prolific as to almost hide the pale nucleus from view. With mixtures of methylene blue and eosin the granules stain with the latter. With Leishman's stain the cytoplasm exhibits a number of larger granules which take on the same colour as the nucleus.

Eosinophile myelocytes.—These cells are as large as the previous variety, occasionally larger; small forms are often present. The nucleus is similar, being spherical, reniform, or crescentic, and with the same structure. The most characteristic feature of the cells is its cytoplasm, densely studded, as it is, with brilliant, strongly oxyphile granules. They stain with the triacid mixture from shades of pale orange to a deep reddish brown; with eosin a brilliant red.

For the study of these cells I have used blood from myelogenous leukæmia and marrow fixed in Fleming's solution, embedded in paraffin and cut into sections. By these means the coarse eosinophile myelocyte exhibits a characteristic architecture.[1] The cytoplasm is seen to contain a denser portion or archoplasm with a centrosome and a reticulum associated with large granulation. On careful examination it will be seen that there is a definite arrangement of the granules, which radiate from the archoplasm over the surface of the cell like meridian lines on a globe. Each granule appears spherical, and stains more deeply at the periphery than the centre; many of them appear as cross sections of a fibril, or are attached by the latter to the archoplasm. Cells stained with Heidenhain's hæmatoxylin, and counter-stained with orange G, eosin or acid fuchsin give good pictures. Basic fuchsin for the nucleus, with orange G for the cytoplasm, is excellent. In sections of marrow which were stained first with thionin blue, then with acid fuchsin and orange G, the nuclei stained blue and the cytoplasm in variations of red and orange. In myelocytes the archoplasm stained well with the orange G, but in many cells I noticed a definite deposit in the archoplasm of fuchsinophile granules, in some few, in others numerous, and gradually increasing in size and affinity for the red dye. By this means a definite transition

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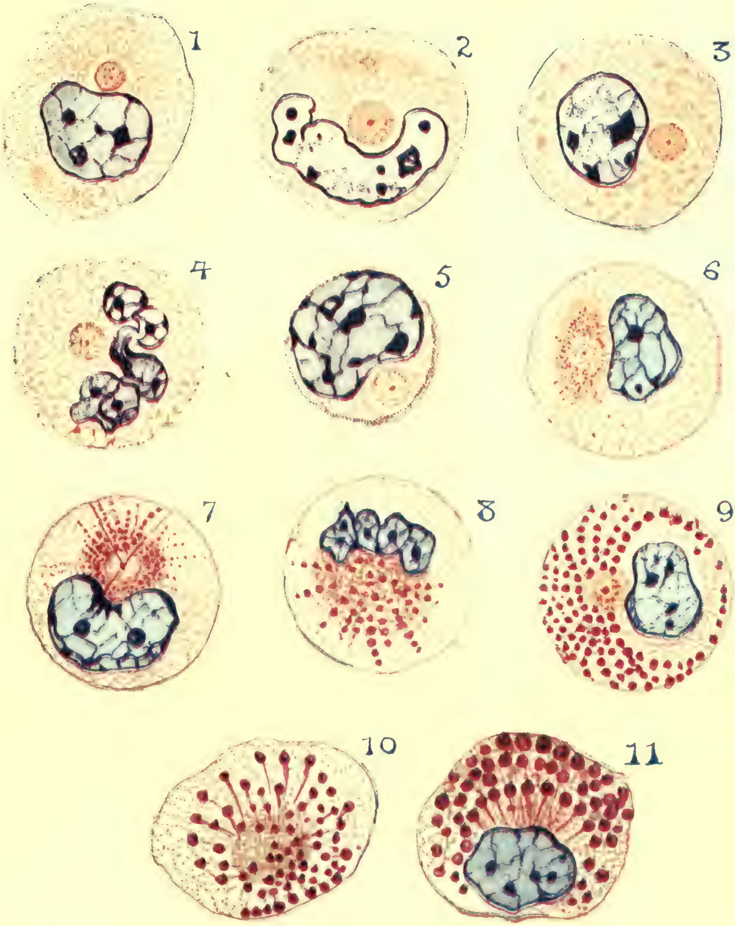
[1] Buchanan, *Liverpool Medico-Chir. Journ.*, Jan., 1908, vol. xxix, p. 178.

PLATE X.



Buchanan

from the myelocyte to the coarse eosinophile cell could be followed, and that the origin of the granulation commenced in and radiated from the archoplasm was determined. In dividing cells I have noticed that the granules take parallel longitudinal lines during the late anaphase of mitosis.

Basophile myelocytes.—These cells contain large basophile granules, the γ granule of Erlich. The cells vary much in size, from that of a lymphocyte to slightly larger than a "polymorph." The nucleus may be spherical or lobulated, situated excentrically and staining so feebly with basic dyes that it is rendered almost invisible by the densely staining basophile granules, so that many cells appear simply as congregations of these granules. The term "mast cell" has been applied both to the fine basophile cell of normal blood and the basophile myelocyte; it is not always an easy matter to distinguish between them. The granules do not react to the triacid mixture, but are well defined by dahlia, Leishman's or Jenner's stain. They appear either deep blue, or show the crimson purple metachromatic reaction. The granules are never all the same size in any one cell; some are small, others larger even than the coarse eosinophile granules. As they vary much in size, so they do in shape, being spherical, oval, triangular or cuboid, and massed together in clumps. From the irregular and angular granulation many of the cells have a spiked appearance by reason of the granules projecting beyond the surface. As a rule they congregate about the periphery of the cell, and in my opinion are situated on the surface of the cytoplasm. The latter is so delicate that in the ordinary blood film it is practically invisible.

Stained by methylene blue and mounted in iodine gum, these granules appear in various shades of brown to almost black.

Myelocytes with mixed granulation.—Large myelocytes may occasionally show both varieties of granulation at the same time. My attention was drawn to this fact in 1896[1] while studying the cytology of the blood in leukæmia.

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[1] Buchanan, *Journ. Path. and Bacter.*, Dec., 1896.

Many of these cells exhibit fine basophile granulation only, even when stained with both methylene blue and eosin used separately. Preparations in which either stain was used alone showed characteristic reactions. Further, films exposed to a mixture of methylene blue and eosin in aqueous solution, washed in water, and then to the action of methylene blue alone, were peculiar in that many of these cells exhibited double granulation; oxyphile and basophile occurring in the same cell side by side. The granules being finer than usual and closely packed gave the cells a violet tint. In cells which have been ruptured or "spread" the different granules can be easily distinguished. These basophile granules, however, are said not to be of the same nature as those of basophile cells, as they do not stain metachromatically. This mixture of granulation seems rather to point to a phase in the nutrition or age of the cell. According to Erlich, the younger the cell, the more its granules tend to stain with a basic dye. I have never seen cells with this so-called mixed granulation in normal blood.

Myeloplaxes (Megakaryocytes).—In instances of extreme leukæmia of myelogenic origin one may meet with cells twice to four or five times as large as the ordinary myelocyte, I have seen them in two cases. Although not so large as the great giant myeloplax of the bone-marrow in other characteristics, they are identical. The nucleus is massive and much convoluted or lobulated, occupying the greater part of the cell, and in not a few instances surrounded by a very scanty amount of cytoplasm. It presents a well marked network with wide meshes, especially in sections of blood fixed in Fleming's solution and stained with hæmatoxylin. Scattered throughout the network are masses of chromatin of varied sizes. No nucleolus is visible. The cytoplasm is made up of a fine reticulum.

Small neutrophile pseudolymphocytes.—These are small cells about the size of a lymphocyte with a spherical nucleus which reacts strongly to basic stains and a small amount of cytoplasm in which a few neutrophile granules are present. These cells are extremely rare, they were described by Erlich in a case of hæmorrhagic small-pox. They are said to be due to fragmentation of the "polymorphs" in the blood, but have also been regarded as a very small variety of myeclocyte.

"Irritation forms" (*Turk's "Reizungsformen"*).—These are described by Turk as mononuclear non-granular cells. They occur in the same pathological conditions as and associated with myelocytes. The nucleus is large in proportion to the size of the cell, deficient in chromatin and stains feebly. The cytoplasm is non-granular and with the triacid mixture stains a deep brown. They have been regarded as an early stage in the development of the erythrocyte. They have been noted as present in the blood of the post-typoid anæmias of infancy by Da Costa.[1] What their clinical significance is remains to be determined.

Leucoblastic cells.—These cells are of special interest and a good deal of controversy has arisen as to their correct classification. They occur in cases of acute lymphoid leukæmia, and are the distinguishing feature in the blood of this disease. They have been regarded as large lymphocytes with an origin from the lymphoid tissues. I do not consider that this idea is any longer tenable. They differ in many ways from the lymphocytes of normal blood, and my personal experience of them leads me to conclude that they are cells a stage further back than the myelocyte. In fact that they are the forerunners of the latter, and develop into it. They are found in all acute leukæmias, but also in the chronic myelogenous leukæmia. In the early stage of the latter disease they may be few in number but as the disease progresses they may become more numerous than the neutrophile myelocytes. When both varieties of cell exist at the same time, the disease has been classified as a "mixed" variety. I do not consider that such "mixed" leukæmias have a double origin, but rather the condition points to a progression of the disease. In one case as it approached a fatal termination the leucoblasts became very evident, and in another a complete change took place, so that instead of the blood picture being one of ordinary myelogenic type, it became one of acute lymphoid (leucoblastic) leukæmia, with a rapid termination, both blood and marrow being loaded with the leucoblastic type of cell.

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[1] *Clinical Hæmatology,* 1902, p. 172.

In the disease known as chloroma this cell is the most conspicuous element both in the blood and marrow. It is a question then if any leukæmia does really originate from the lymphatic system, and I consider that acute lymphoid leukæmia is of myelogenous origin, and that the cells which are so characteristic of it are not lymphocytes, but leucoblastic cells derived from the bone-marrow. They are cells which give rise to myelocytes, and their presence in the blood indicates a profound disturbance of the marrow in a retrograde direction.

Leucoblasts vary in size, some are smaller than a "polymorph," others as large as the typical neutrophile myelocyte. They are usually spherical with a large nucleus almost filling the cell, so that in some only a very thin rim of cytoplasm can be seen. The nucleus is finely reticulated and poor in chromatin, so that it stains feebly. One or two small vesicular-looking nucleoli may be present in it. As a rule the larger the cell the paler the nucleus. The surrounding cytoplasm is finely reticulated, and with basic stains appears darker than the nucleus, and composed of a closely-packed fine basophile granulation with an occasional vacuole; this appearance is well seen when the cells have been stained with Leishman's stain. With the triacid mixture the cytoplasm is coloured a delicate pink, and in some cells a development of neutrophile granules takes place. With methylene blue alone the cytoplasm is faintly stained, but with eosin as a contrast stain, fine oxyphile granules can be seen in many cells. The youngest variety, it would seem, has a closely reticulated basophile cytoplasm stretched tightly round the nucleus. The latter, as the cell develops, becomes excentric and reniform, the cytoplasm more abundant and less responsive to basic stains, thus appearing as a non-granular myelocyte in which the development of specific granulation determines the class to which in future it will belong. As the nucleus becomes reniform a clear area is seen in the bay, containing a single centrosome.

The leucoblast is a delicate cell easily broken in the preparation of a film, so that only a mass of nuclear material may be seen. In the fluid exudate from the pleural cavity in a case of acute lymphoid leukæmia, I found these nuclear masses were very numerous. Many of the leucoblasts show protuberances from the surface. The cytoplasm is said to give a much feebler alkaline reaction with iodine-eosin than that of the lymphocyte.

The Nature and significance of the granules.—Several theories have been promulgated in regard to the nature and function of the granules of leucocytes, with evidence in support of each. Erlich[1] regarded them as specific metabolic products of cellular activity, to be given off by them, not as waste material, but for a special purpose. Altmann[2] held that they "serve as a foundation for the explanation of many phenomena of organic metabolism, that they effect both reduction and oxidation by means of the transference of oxygen, and of thus effecting the decomposition and combination of bodies, without losing their own individuality."

There seems to be much evidence that these granules are of a specific nature and function, by their histo-chemic properties, their structure and their relation to the age and nutrition of the cell. Not only does the mature cell exhibit a granulation peculiar to it, but they vary in different species of animals. The coarse eosinophile granules are albuminous and contain iron, they have a definite structure in different animals. In man they are spheres; in birds they occur in two forms, one of which is crystalline; in other animals they are cuboid, and the largest forms of all are found in the blood of the horse. I have already pointed out their relation to the archoplasm of the cell in man, and their peculiar arrangement in the cytoplasm, being part of the cytoreticulum. It is peculiar also that granulation is most marked in cells with amœboid activity; Kanthack, Hardy and Hankin, attributed to them bactericidal properties, that they exert a deleterious influence upon invading organisms in their vicinity and discharge their granules amongst them for this purpose. In tubercle I have noticed an accumulation of eosinophile cells as a surrounding cordon, the granules collected upon the side of the cell nearest to the tubercle. The special response made by cells containing certain granules to specific stimulations adds weight to the probability of their being endowed with definite functions.

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[1] *Farbenanalytische Untersuchungen*, xii, p. 134.

[2] *Die Elementarorganismen.*

Amœboid activity and phagocytosis of leucocytes.—In a specimen of fresh blood examined on a warm stage at the body temperature, changes of shape and position may be observed in many of the leucocytes. These alterations are closely associated with their power of engulfiing foreign particles or infecting bacteria. Metschnikoff[1] by his brilliant researches into this cell function, and which he called *phagocytosis*, established his theory of immunity or power of the body-cells to resist infective processes; and although more recent investigations have led to the discovery of the part played by the serum in relation to immunity, and so to a modification of his original theory, phagocytosis still stands as a great factor in safeguarding the body from outside invasion. Amœboid leucocytes may alter in shape or position by their innate power of contractility and expansion. The movement may be limited to the slightest alteration of contour, or so extensive as to result in locomotion. A cell with marked activity may be seen to protrude a portion of its protoplasm in a certain direction and then gradually move along it, or it may protrude several such pseudopodia which may stretch out several times the diameter of its body in the form of flagella. Amœboid movement may go on for many hours. I have watched it for twenty-six hours in cells of a single specimen. When phagocytosis is proceeding, the cell flows round and takes the particle or organism into itself. Leucocytes do not exhibit phagocytic action to all organisms alike. In some experiments[1] made by me in 1898 with hang-drops of leukæmic blood inoculated with typhoid and anthrax bacilli, phagocytosis was pronounced towards anthrax, but it did not take place in the typhoid drops. With typhoid bacilli, half an hour after preparation there was marked clumping of the bacilli and loss of their movement. There was good amœboid movement. Four hours later the bacilli had again become very motile, amœboid movement had stopped, and there was no evidence of phagocytosis. The cell granules had become very agitated, and many cells were seen to be already dying. Five and a half hours later there was no cell movement, no seething of granules and no phagocytosis. Ten hours later the leucocytes had become quite round. Although no phagocytosis occurred, the leucocytes as it were being paralysed, the amœboid movement of the early stage was good. The first to take place was granular agitation, then frequent alteration on the part of the cell. A pseudopodium would be thrown out to a distance measuring three-quarters of an inch of the microscopic field stretching round several red corpuscles, to reach a bacillus, but no attempt made to enclose it. With anthrax, amœboid movement and phagocytosis were both active, and in cells adjacent to bacilli the granules were always agitated. Of the leucocytes present amœboid movement and phagocytosis were exhibited by the neutrophile, hyaline and coarse eosinophile cells of the blood. Myelocytes, both neutrophile and eosinophile, were inactive.

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[1] *Comparative Pathology of Inflammation.*

Under certain conditions the neutrophile cells of the blood may become phagocytic to the erythrocytes.

By reason of their amœboid activity leucocytes are enabled to leave the vessels by diapedesis during inflammatory processes or in health.

Foremost of the leucocytes in amœboid and phagocytic activity is the polymorphonuclear neutrophile. It is endowed with both functions in high degree. This can be admirably studied by the method of incubating leucocytes and bacteria in the presence of serum in finely drawn pipettes, used for determining the "opsonic" content of the blood.[1] During this process I have noticed that the nuclei of the "polymorphs" show a peculiar alteration of shape. This, to a degree, depends upon the length of incubation, and is best seen in those in which phagocytosis has been allowed to proceed longer than the usual quarter of an hour. Two hours has been the extreme period of incubation covered by the experiments in which the nuclear alteration was noted. This change of shape results in the division of the nucleus into many parts, but each attached to the other by a delicate thread. Thus the polymorphic

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[1] *Liverpool Med. Chir. Journ.*, 1898.

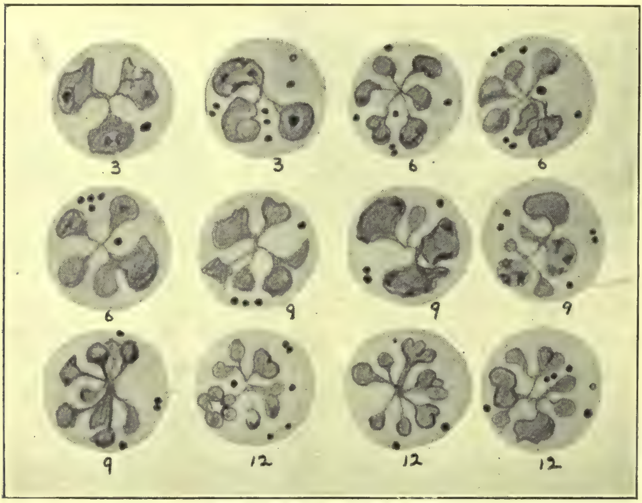

FIG. 29.—NUCLEAR ALTERATION IN POLYMORPHONUCLEAR LEUCOCYTES DURING PHAGOCYTOSIS. (R. J. M. Buchanan.)

Reading from left to right :—
Line 1. 1 and 2 are divided into three lobes.
3 and 4 into six lobes.
" 2. 1 is divided into five lobes, one of which is equal to two.
2 is divided into five lobes, three small and two large, equal to three each = 9.
3 : four divisions, two of three, one of two, and a single one = 9.
4 : six lobes, three small ones and three large, equal to two small = 9 (or three large, equal to three small = 12).
" 3. 1 : nine small divisions.
2 : six lobes, three small, two equal to two, one equal to five = 12.
3 = seven lobes, four small, one equal to two, two equal to three each = 12.
4 = " " " " "
Drawn from the film examined, × 1,500 diameters.

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[1] *Liverpool Med. Chir. Journ.*, Jan., 1908, vol. xxix, p. 12.

character is retained, but greatly increased. Generally the nucleus has an average of three large lobes, but each may divide and subdivide in such a way that the nuclear content may be estimated as multiples of three. Thus if there were originally three large masses and one divide, the division is unequal, so that one portion of it is equal in content to two of the other. That the nuclear content is polyvalent and expressed in this way appears to me the correct interpretation of this phenomenon. It results in the alteration of the nucleus into bizarre designs. When it has proceeded to a division into twelve parts, these are generally arranged at the periphery of the cell, and connected by threads to the centre, forming a rosette. In only one instance have I noted more than twelve parts, counting twenty one. The larger portions are generally more deeply stained than the smaller. A fully rosetted nucleus stains very lightly. Why such changes in shape occur is a difficult problem to decide. It is evidently associated with amœboid activity, allowing a quicker, more active, and complicated movement of the cell for purposes of phagocytosis. I could not discover any relation between the number of nuclear divisions and the number of cocci engulfed by a cell.

The large mononuclear (hyaline) cells are both amœboid and phagocytic.

Coarse eosinophiles are actively amœboid and to a certain extent phagocytic.

Lymphocytes are regarded as able to change their shape, but not locomotive. They are not supposed to be phagocytic, but I have noted on several occasions when incubated with tubercle bacilli that several may congregate around a group of bacilli, and the cytoplasm adjacent to the latter show considerable irregularity of outline. The hæmic basophile cell is amœboid; neutrophile and eosinophile myelocytes are regarded as slightly amœboid, but sufficient for their emigration into the circulation from the marrow. I have noticed sluggish movement of eosinophile myelocytes on the warm stage, but not neutrophile. The motility of the basophile myelocyte has not been established.

Dr. Mary Rowley, of Boston,[1] records an extraordinary case of anæmia in which active phagocytosis was carried on in the patient's blood by every variety of leucocyte. In this case not only were the usual blood cells (polynuclear leucocytes, lymphocytes, eosinophiles and mast cells) engaged in phagocytosis, but also the myelocyte and another type of cell, similar to that which has been described as a "plasma cell." The most active cell was the large mononuclear, or as Dr. Rowley terms it, the "large lymphocyte." The cells were actively engaged in phagocytosis in the peripheral blood, engulfing not only erythrocytes, but also each other. So active was the phagocytosis that had all the leucocytes been engaged in the process, when the leucocyte count was 100,000 per c.mm., they could have destroyed all the erythrocytes in the body within two hours. The leucocytes exhibited extraordinary vitality, and when removed from the body preserved their powers of motion and phagocytosis for weeks and even months.

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[1] *Journ. of Experimental Medicine,* vol. x, No. 1, Jan., 1908.

Effects of Citration upon leucocytes—flagellation.—In specimens of blood from myeloid leukæmia which were several times washed and centrifuged in 0·5 per cent. sodium citrate in normal saline solution, then very carefully spread into films and stained with Leishman's stain, I have noticed most peculiar alterations in contour, and which I regarded and described as flagellation. From the surface of the large neutrophile myelocytes, and also from the other varieties of mononuclear cells, fine prolongations extended in a form resembling flagella, and which were thicker at their attachment to the cells than at their extremity (Fig. 30*b*). From some cells only one, from others several flagellate prolongations were noted. When situated away from the nucleus they were formed of cytoplasm only. When derived from the cell in close proximity to the nucleus, fine strands of nuclear chromatin substance extended from the nucleus for a short distance into them. In cells containing "ruby" granules, these were occasionally found at the base of these flagellate prolongations. These flagellate prolongations varied in length from one to several times the diameter of the cells. They were clearly distinguished from pseudopodia such as one sees in the actively amœboid polymorphonuclear cells, and as they occurred in the absence of organisms they were not associated with active phagocytosis at the time. In relation to a paper on the etiology of cancer read before the Liverpool Medical Institution in 1899, I exhibited specimens and illustrations of a similar phenomenon which I had noted in connection with the cells forming the round cell infiltration occurring in the proximity of minute secondary deposits in the interstitial spaces in sections from a case of carcinoma of the stomach. Large numbers of the round cells had lost their usual spherical shape, and appeared markedly spiked and irregular (Fig. 30, *a*, *a′*). From their surfaces projected fine prolongations, to the ends of which were attached minute oval or diamond-shaped spore-like, refractile bodies, which stained brilliantly with the methyl green of the Erlich-Biondi-Heidenhain stain. They also stained well with Erlich's acid hæmatoxylin; in fact, they responded to stains as does chromatin, particles of which I concluded they were. They resembled miniature spermatazoa, but whether they were in any way connected

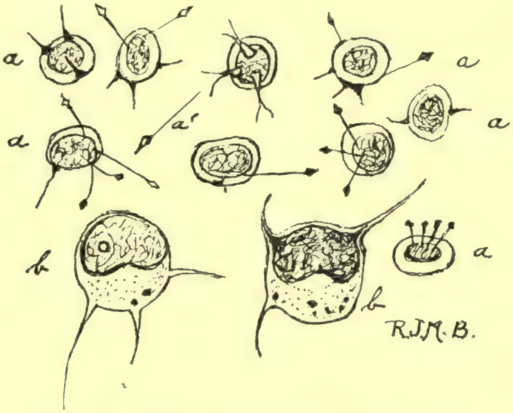

FIG. 30.—FLAGELLATION OF LEUCOCYTES.

a—Round cells from carcinoma of stomach showing flagella with heads like spermatazoa.
a′—Free flagellum.
b—Myelocytes from leukæmic blood with flagellate prolongations, after citration. (R. J. M. Buchanan.)

with cell fertilisation it was impossible to determine. Some of these flagellate bodies were found free from the cells. Such a conjecture reminded me at the time of the theory promulgated by Klebs some years before and mentioned by Ruffer and Walker in their work on the so-called parasites of cancer,[1] that one of the functions of leucocytes was the fertilisation of cells throughout the body. Their appearance gave support to a conviction that I have always had, that, as cell comes from cell, so each must be fertilised to ensure reproduction. This I regard as a definite law, not proved, may be, by actual observation of the phenomenon, but recognized by constant recurrence of effect.[2]

[While this work has been passing through the press, H. C. Ross and C. J. Macalister have recorded interesting accounts of their personal investigations on the flagellation of lymphocytes in the presence of excitants both artificial and cancerous. The reader is referred to their published articles: *Brit. Med. Journ.*, Jan. 23, 1909, p. 206, with bibliography.]

IODOPHILIA

If a film of normal blood be exposed to the vapour of iodine before it has time to dry, many leucocytes will be seen to contain granules which are stained a mahogany brown colour with the iodine. Further, free extracellular particles of varied size and shape which give a similar reaction are present in the plasma. This "vital iodine-fixation" does not occur in normal blood if the film be allowed to dry prior to its exposure to the iodine vapour. If such a reaction occur in a dried film it denotes a pathological condition.

If dried films of normal blood be treated by iodine vapour and mounted in lævulose syrup, or cedar oil, or mounted direct in iodine-gum solution, the leucocytes will be stained a faint canary yellow to the exclusion of the nucleus, which remains unstained and refractile so that its shape can be clearly defined and the type of leucocyte recognized. In certain pathological conditions, however, the leucocytes exhibit the iodophilous reaction. It occurs principally in the polymorphonuclear neutrophiles, to a less extent in lymphocytes and myelocytes; yet more rarely in eosinophiles or basophiles.

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[1] *Journ. Path. and Bact.*, 1892.

[2] *Liverpool Medico Chir. Journ.*, Feb., 1900, p. 156.

The intracellular reaction may occur in three ways, either by a diffuse brown staining of the cytoplasm, by the manifestation of granules of varied shape and size in it, or by a combination of both in the same cell. The granules may be situated near the nucleus or in separate groups, less often at the periphery of the cell. Coarse granulation is more often seen in lymphocytes and eosinophile cells.

The free extracellular deposits in the plasma may be spherical, oval or irregular in shape. Some are associated with fragments of leucocytes, and there is no doubt that many of them are blood platelets. The clinical significance of the extracellular iodophilous deposits has not been determined.

The nature of the granules has been a matter of some dispute. It is the generally accepted opinion that they consist of glycogen, which in pathological conditions has become more insoluble and easily demonstrated.

The clinical significance of leucocytic iodophilia rests upon the fact that it is a constant feature in all conditions associated with suppuration and toxæmia, unless the pus be so walled off as to prevent absorption of toxic material. It is present in diabetes, in septic conditions of all kinds, in pneumonia, tubercular mixed infections, malignant disease, pernicious anæmia, leukæmia, and gonorrhœal arthritis. According to Locke and Cabot "no septic condition of any severity can be present without a positive reaction."[1] There is, however, no direct relation to the degree of leucocytosis, anæmia, or pyrexia. (For a full account of iodophilia the reader is referred to papers by J. C. Da Costa reporting the investigation of 100 cases, in the *Therapeutic Gazette*, October 15, 1906, and one by J. Barnicott, with conclusions based on the examination of over 200 bloods, in the *Journal of Pathology and Bacteriology*, Vol. XI. No. III. June, 1906.)

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[1] *Jour. of Med. Research*, Bost., Jan., 1902, vol. vii, p. 25.

SUDANOPHILIA

The presence of fat granules in the leucocytes may be demonstrated by staining films with a 0·2 per cent. solution of Sudan III in absolute alcohol. It stains the granules brick red. In conjunction with this a 0·1 per cent. alcoholic solution of brilliant cresyl-blue is used. This method is advised by Cesaris-Demel, Buttino and Quarelli.[1] The sudanophile granules are increased in all suppurative conditions and pneumonia. The percentage of sudanophile leucocytes is high in purulent meningitis, above 50 per cent.; in tubercular meningitis it is generally low, under 16 per cent. These granules may be due to products of leucocyte degeneration or fatty materials which healthy cells have taken up.

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[1] *Rif. Crit. di Clin. Med.*, Florence, 1907; *Brit. Med. Journ.*, August 24, 1907.

CHAPTER VIII

The Origin of Leucocytes—Leucocytosis and Leucopœnia—The Nature of Leucocytosis.

THE ORIGIN OF LEUCOCYTES

Of all the problems associated with the study of hæmatology none seems so difficult of solution as the origin of leucocytes. It would be rendered comparatively easy, if by experimental removal of matrices which are regarded as birth-places of these cells one could judge the effect upon the leucocyte picture as a whole. These matrices do not lend themselves to such experimental methods, so that with but few exceptions, deductions have to be made from clinical and pathological conditions and the blood disturbances associated with them.

In early embryonic life the blood contains a few lymphocytes only. At about the fourth month neutrophile and eosinophile myelocytes, polymorphonuclears, lymphocytes and non-granular cells with tri-lobed nuclei are present (Engel). Before leucocytes are found in the blood stream primary wandering cells are found collected in various situations in the tissues, particularly the embryonic liver. According to Beard [1] the rudimentary thymus is the birth-place of leucocytes prior to the formation of the spleen or lymphatic tissue. Gulland [2] also found the earliest collections of leucocytes in the vicinity of the thymus. In post-natal life the chief matrices regarded as seats of leucocytic formation were the marrow, lymph glands and spleen. It is generally accepted that the last of these has really no association with the origin of leucocytes in man. Congenital absence and the effects of extirpation of it produce no alteration of the blood state that would point to the spleen as an active agent in leucocyte formation. After splenectomy an increase of lymphocytes takes place for a short time, and only after a year or two does a certain degree of eosinophilia follow.

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[1] *Anat. Anzeiger*, 1900.

[2] *Journ. Physiology*, vol. xix, 1896.

The two main sources of leucocytes are the bone marrow and the lymphatic nodes throughout the body. Much labour has been expended and many views put forward regarding the question of a primary " mother " cell remaining after birth which begets both erythrocytes and leucocytes. On the one hand it has been contended that the primary wandering cell of the embryo persists, and in the adult is the progenitor of coloured and colourless cells. On the other, that erythrocytes and leucocytes are developed from a separate series of cells which have become differentiated from the embryonal ancestral cell. Leaving the erythrocyte out of the question at present, one is faced with the problem of the origin of leucocytes in the adult. Because of the myelogenous and lymphogenous origin of leucocytes, two schools of teaching have arisen, those of Erlich and Uskoff; Erlich,[1] holding the view that lymphocytes are developed in the lymphatic tissues throughout the body, and the other leucocytes of normal blood exclusively from the bone-marrow, that they are in no way related to one another and remain as two distinct groups of cells to be classed separately; Uskoff[2] and his followers contending that the lymphocyte is the parent cell of all leucocytes.

Each have had their supporters. Erlich rigorously defended his position, and until more convincing evidence can be produced in favour of the lymphocytic origin of all leucocytes his views must still remain tenable. The rôle of the so-called " lymphocyte " of lymphatic leukæmia has been largely responsible for the idea that from lymphocytes all leucocytes evolve. In cases of lymphatic leukæmia the marrow has been found so changed as to consist largely if not wholly, of lymphocytes so-called. The trend of opinion is toward the idea that the cells of acute leukæmia are not lymphocytes at all, and are different from the lymphocytes of normal blood. I am quite of this opinion. If this cell be other than a lymphocyte, it will be necessary to give it a place in the classification of leucocytes. It is the cell which in the previous table is termed a "leucoblast." It is present in acute leukæmia as the predominating element, occurring in the "mixed" leukæmias, and as I have previously noted in one case, at first with an ordinary blood picture of myeloid leukæmia, this cell eventually replaced all others to such a degree that the blood picture became one of acute leukæmia, with the same appearance in the marrow. In acute leukæmia this cell may be the preponderating element in the blood and marrow, without any marked alteration in the lymphatic glands. In chloroma it is numerically the chief cell in both blood and marrow.

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[1] Loc. cit.

[2] *Archiv. des Sciences Biolog.*, St. Petersburg, 1893–97.

The considerations of such conditions point to the great probability of this leucoblastic cell being the original one from which others are derived—the hæmic lymphocyte excepted. These cells have their origin in the bone marrow. Pappenheim regards them as lymphocytes, Nägeli [1] termed them "myeloblasts" and recognised differences between them and hæmic lymphocytes; Grawitz [2] and Wolff [3] also consider them as "mother cells," and capable of being transformed into the leucocytes of the marrow. Erlich also regards them as the original cells from which spring the large myelocytes. Some authorities consider these primitive lymphoid cells as progenitors of all leucocytes, lymphocytes included; but further, that from them hæmoglobiniferous cells may also be derived; thus begetting or developing on the one hand erythrocytes, on the other leucocytes.

Leaving out the gross changes recognized clinically in organs such as the spleen and lymphatic glands, the variations of the blood picture as revealed by the microscope afford a basis for classification of abnormal blood states which is now considered the most rational and trustworthy. To irregularities of the dual function of the parent cell on developmental lines, the various abnormal blood states (of a persistent character) depend. Thus, from a lack of development in the direction of erythrocytes, erythropœnia would follow, with or without the occurrence in the blood stream of erythrocytes not present in normal blood. Excessive development would give rise to polycythæmia. In the leucocyte direction decrease or excess of development would lead on the one hand to leucopœnia, on the other to a leucocytic increase of such a nature as occurs in the various leukæmic states. Irregularity of both functions at once would be responsible for the production of the peculiar condition known as leukanæmia, in which the blood picture may be at one time that of pernicious anæmia, at another leukæmia, or both combined. It must, of necessity, be expected that these two functions be so governed as to ensure an equilibrium of activity for the determination of a normal blood state. Whether loss of activity of the one produces, follows, or is in any way related to an increased activity of the other, has yet to be determined. It can hardly be accepted as an established and uncontrovertible fact that these cells are endowed with any such dual capacity, or that they alone are the undoubted primary source of leucocytes, apart from erythrocytes. Too little attention has been paid to a careful study of the large giant cells of the marrow for the purpose of investigating the possible position they may be found to hold in the genesis of erythrocytes or leucocytes. These colossal cells, with their peculiar reticulated cytoplasm, branching out from them to produce fine processes, joining one to the other to form a great organic syncitium, housing as they do great masses of nuclear material, appeal to me as the most probable source from which all marrow-born leucocytes are derived. Their method of division is two-fold; both direct and indirect. Although the latter is the usual way amongst the general tissue cells of the body, in the leucoblasts both processes are known to take place. Löwit,[1] Saxer,[2] and Arnold[3] have all upheld the view that direct division does occur in leucocytes, and it seems to be a significant feature of the life history of the giant cells of the bone marrow. I have studied this in sections of human marrow fixed immediately it has been removed from the living body, and find the giant myeloplaxes dividing in different ways; some by the regular method of mitosis, others showing modifications in the form of multipolar mitosis, and others presenting all the appearances of amitotic multiple division. In the latter, portions of nuclear material are to be seen budding off from the main mass, some lying isolated in the cytoplasm, others at the periphery passing out from the cell body. I regard these nuclear buds as most probably the initial stage of the primary lymphoid or parent cell, which may, in the future, be shown to develop into erythrocyte and leucocyte.

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[1] *Deutsche med. Wochenschr*, No. 18, 1900.

[2] *Klinische Pathologie des Blutes*, 1896.

[3] *Zeitsch. f. klin. Med.*, 1902, vol. xlv.

The investigations of Pugliese[4] into the effects of splenectomy in the hedgehog show that in this animal the splenic loss is followed by a marked increase of the giant cells of the bone marrow, and he claims that these become transformed into leucocytes by amitotic division. It is reasonable, therefore, to attempt a compromise between the two opposite opinions of Erlich and Uskoff. By the transference of the "leukæmic lymphocyte" to the myelogenous series of cells, Erlich's original position might be accepted to the extent that the leucocytes of normal blood arise from two main sources: the lymphocytes from the lymphadenoid tissues; and all the others, amongst which must be included the "leukæmic lymphocyte" or "leucoblast" from the bone marrow. On the other hand, so far as bone marrow may be regarded as a lymphoid structure, the parent cell may be considered as giving rise to all leucocytes, lymphocytes included, and that the latter may be a terminal developmental stage of one variety of cell. It

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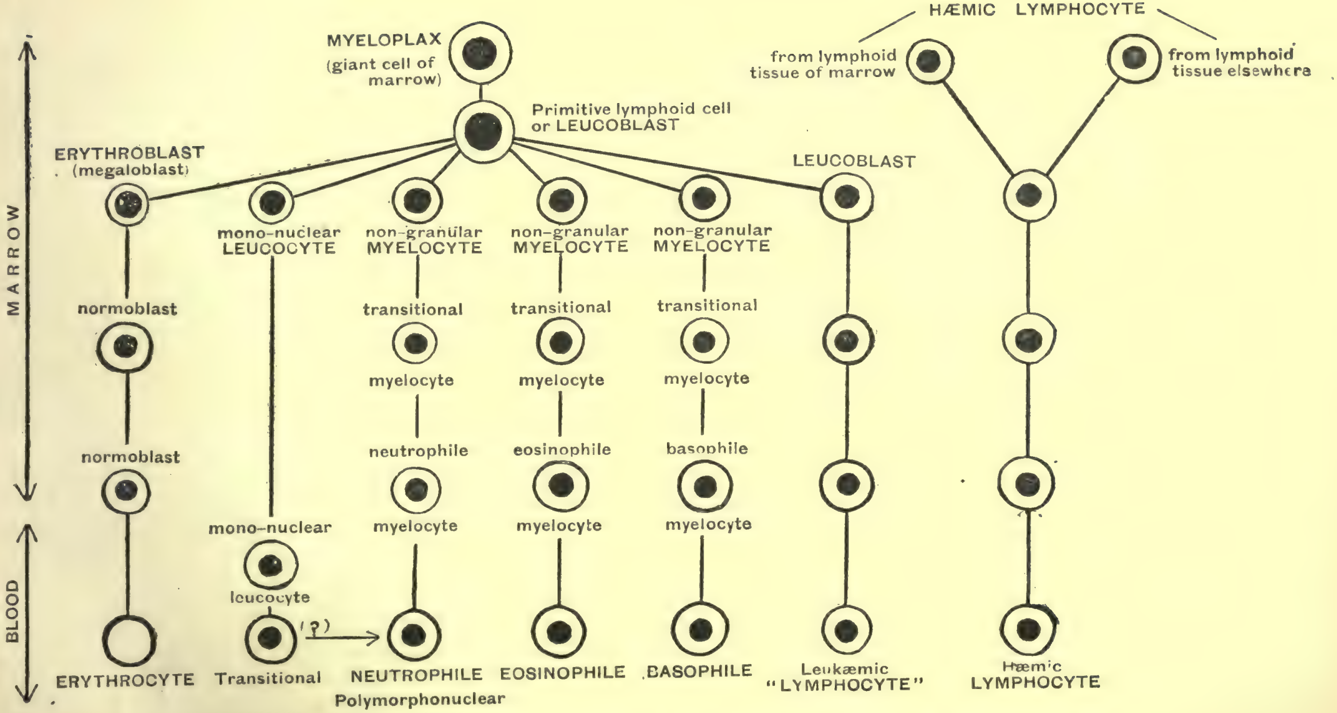
[1] *Archiv. f. mikr. Anat.*, vol. xxxviii, 1891.

[2] *Anat. Hefte*, vol. vi, 1896.

[3] Virchow, *Archiv*, vol. cxl, 1895.

[4] *Fortschr. d. Medicin*, vol. xv, No. 19, 1897.

FIG. 31.



SCHEME OF POST-NATAL GENERATION OF ERYTHROCYTES AND LEUCOCYTES does not seem satisfactorily established that the hæmic lymphocyte is the young form of the other leucocytes. Until pathological blood states associated with an increase of mononuclear leucocytes of the lymphocyte type have been clearly differentiated and the cells relegated to their proper places and genesis, the subject will continue to present many difficulties.

The development into the adult leucocytes may be followed by reference to the plan laid down (Fig 31). The non-granular myelocyte becomes neutrophilic and gradually changes to the characteristic neutrophile myelocyte, from which by alteration and condensation of the nucleus a transition follows to the polymorphonuclear neutrophile leucocyte of the blood. In the same way, others gradually become eosinophile and form the eosinophile myelocytes, and by a similar transition to the preceding become the eosinophile cells of the blood. The basophile leucocytes of the blood most probably are derived in a similar manner from basophile myelocytes. It is not quite certain what the undoubted origin of the large mononuclear cell of the blood is, whether from lymph gland, spleen, or bone-marrow, but there is reason to consider that it is derived from the latter. The transitional cell of the blood is supposed by Erlich [1] to be an intermediate stage between the large mononuclear and the neutrophile leucocyte. That it is so has not been absolutely determined. I consider there is reason to believe that many of them are transitional forms between the neutrophile myelocyte and the neutrophile leucocyte.

When the different leucocytes have reached their full development, there is little or no reason to believe that they are ever transformed or pass over from one group to another. It is most improbable that a neutrophile cell should ever become a basophile cell, or an eosinophile leucocyte a basophile or neutrophile, with such marked differences of morphology and function. I do not think such a change ever takes place, but that each cell retains its individuality until its death.

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[1] "Diseases of the Blood," *Nothnagel Encyclo.*, p. 66.

LEUCOCYTOSIS

The term **leucocytosis** is used to indicate an increase above the normal number of leucocytes present in the blood stream. As an index of the normal is the number usually present in a cubic millimetre of blood. This may vary in health from 5,000 to 10,000. As a rule, any excess above 10,000 per c.mm. is regarded as a leucocytosis, but it does not really indicate that the total number of leucocytes in the blood is raised. Certain factors may be at work which give false indications. Any diminution in the amount of plasma would give the impression of a leucocytic increase in the amount of blood examined; there might be a diminished physiological loss, with a normal production, or the production increased and the loss normal; or the excess may be due to a local determination of the cells to the part whence the blood is taken, or the cells might multiply in the blood stream, a rare occurrence in adult cells. The reverse of these conditions would give a false indication of leucocytic decrease or leucopœnia.

As there is generally an increase at certain periods of the day, and after meals, the count should be made always at the same hour of the day. The average number of leucocytes varies in different individuals in health, so that, when possible, the average for the individual should be obtained and the gain or loss reckoned by comparison with it.

Leucocytosis is rarely due to a numerical increase of all the leucocytes in their normal relative proportions to one another; it is generally due to an increase of one kind of cell absolutely and relatively to the others. A relative increase of one variety may be due to a decrease of another, and vice versa. As leucocytosis indicates an increase and leucopœnia a decrease, so these terms may be applied to the leucocytes as a whole or to any one type of cell. The term leucocytosis, however, has by general usage been associated with an absolute and relative increase of the neutrophile polymorphonuclear cells. Any increase of other varieties has been associated with their individual names, an increase of lymphocytes is termed a lymphocytosis; eosinophiles an eosinophilia; basophiles basophilia; myelocytes myelocytosis or myelæmia. Large mononuclears and transitional cells, as mentioned before, are usually classed with lymphocytes. If the so-called "lymphocyte" of acute leukæmia is to be classed separately, the term leucoblastæmia would indicate their presence in the blood stream. When more than one variety is increased, a mixed leucocytosis occurs.

A leucocytosis may be transient or persistent, although usually the former. A polymorphonuclear leucocytosis may continue for a long time, but the persistent type of leucocytosis is usually a mixed one, and as such is generally associated with the abnormal blood states termed leukæmias. Apart from the leucocytosis associated with inflammatory processes and special abnormal blood states, it occurs physiologically, so that there are two main divisions of leucocytosis, **physiological** and **pathological.**

PHYSIOLOGICAL LEUCOCYTOSIS

An increase in the number of leucocytes as a whole, or of one variety only, does occur under certain conditions. Whether there be a true increase in the number, depending on a greater activity of production, or whether it be due to plasmatic alterations or local determinations, is not known. The rise is generally transient and never great. The following are the usual conditions associated with a physiological leucocytosis.

Leucocytosis of the new-born.—The leucocyte count in the new-born is always greater than in the healthy adult. At birth it ranges from 15,000 to 20,000 per c.mm. for the first two days, when a fall takes place, reaching 10,000 to 15,000 by the end of the second week, at which it remains and may be considered normal under one year. It is generally due to a polymorphonuclear gain during the first ten days, then to a lymphocyte gain. It is closely associated with plasmatic alterations and the active digestive processes of early life. After the first year a polymorphonuclear rise again takes place coincident with a lymphocyte fall, up to the fifth year, when the percentages are those of adult life.

Leucocytosis of digestion.—A leucocytosis takes place after meals and during digestion for two or three hours, when it declines. The gain may reach 33 per cent., and may be due to an increase of all the cells in relative proportion, or to the polymorphonuclear, or as occurs in children to the lymphocytes. It is not always present, and depends greatly upon the state of digestion and assimilation; thus when digestion is slow or interfered with it may not take place, or only to a slight extent. In starvation and allied conditions there is usually a leucopœnia, followed by a marked leucocytosis when food is taken.

Digestion leucocytosis appears principally after the mid-day meal.

Leucocytosis of pregnancy and the puerperium.—A slight leucocytosis takes place in pregnant women towards the full term of gestation. Some investigators have found it more marked in primiparæ than multiparæ, others the opposite. A rise may occur on the first day after delivery, followed by a fall to normal by the fourth or fifth day. It is usually a polymorphonuclear gain, but according to Given,[1] a lymphocyte rise takes place about the twelfth day, which he attributes to uterine involution, and considers its appearance of favourable import. Lactation has no effect upon the leucocyte count; any increase may be traced to some degree of mastitis.

Leucocytosis of exercise and the effects of temperature.—A general leucocytosis follows active exercise or massage, either local or general. The same applies to brief exposure to cold, as after cold bathing, or prolonged hot baths or dry heat. Prolonged exposure to cold causes a leucopœnia. The change is usually transient and due to vaso-motor influences.

Terminal leucocytosis.—A leucocytosis takes place just before death, and is especially marked in conditions in which death takes place slowly, and a moribund state has lasted for some time. As a rule, the count shows an increase in the polymorphonuclear cells, but the rise may be in lymphocytes and the blood picture resemble that of lymphatic leukæmia. It is generally due to peripheral blood stasis or some terminal infection.

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[1] *Journ. Obstetrics and Gynæcol. of Br. Emp.*, 1906.

PATHOLOGICAL LEUCOCYTOSIS

In certain pathological conditions an increase in the number of leucocytes takes place, and is generally dependent upon a polymorphonuclear gain; but other varieties are associated with a rise of lymphocytes or the occurrence in the blood of cells not normally present.

As a rule, the numerical increase of such leucocytes as are normally present in the blood stream does not exceed twice the normal, but occasionally it may reach 40,000 to 50,000 per c.mm. In blood states associated with the appearance of cells normally foreign to the blood the count may greatly exceed this figure. In the large majority of cases the rise is due to an increase of polymorphonuclear neutrophile cells, but in certain conditions it may be due to the lymphocytes or eosinophile cells, and very rarely myelocytes and transitionals may appear. As the neutrophile rise takes place there is a relative decrease in the percentage of lymphocytes and eosinophiles, and the latter may even disappear.

The varieties of pathological leucocytosis are as follows, and are associated with a polymorphonuclear increase.

Post-hæmorrhagic leucocytosis.—A polymorphonuclear leucocytosis follows hæmorrhage from trauma or other causes. It occurs early, persists until the blood is regenerated, and depends in amount upon the recuperative power of the individual. Occasionally a lymphocytosis occurs, or myelocytes may appear in the blood. After traumatic hæmorrhage the leucocytosis persists longer than when the loss of blood is due to other causes.

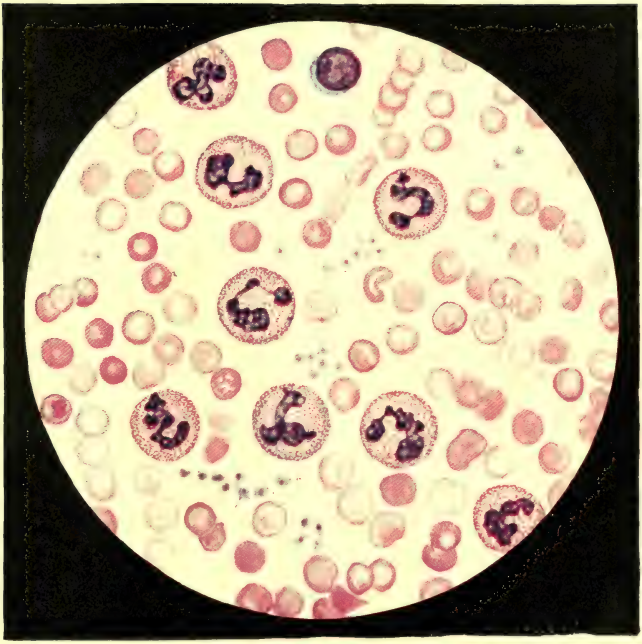
Inflammatory leucocytosis.—Leucocytosis occurs during the course of almost all inflammatory conditions, most of which are due to bacterial infection. It is the expression of an attempt on the part of the individual to overcome the infective causative factor of the inflammatory or infective process. The degree of leucocytosis is an indication of the resistance offered, and when the inflammatory process is productive of purulent exudation the increase, as a rule, is greater than when the exudate is serous. As long as the inflammatory process is active and progressive, the leucocytosis is very evident, but when the inflammation becomes chronic or the process localized and walled-off, then the leucocytosis may be insignificant. The degree of leucocytosis in an individual who reacts well, depends not only on the power of reaction but the severity of the infection. In individuals with feeble resistance and severe infection the leucocytosis will be little or none. Certain infective processes do not invoke leucocytosis whether they be mild or severe; these are exceptions and the fact may be of diagnostic value. In animals in which experimental leucocytosis has been produced, a diminution in number may precede the rise, but this has not been recognized clinically in man.

The following list comprises the conditions usually accompanied by a leucocytosis.

1. GENERAL INFECTIOUS DISEASES.

Actinomycosis.
Asiatic cholera.
Cerebrospinal meningitis.
Diphtheria.
Dysentery.
Erysipelas.
Glanders.
Infective endocarditis.
Multiple neuritis (acute, febrile stage).
Osteomyelitis.
Pertussis.
Pneumonia.
Pyæmia.
Relapsing fever.

PLATE XI.



RJMBuchanan

- Rheumatic fever.
- Scarlet fever.
- Secondary syphilis.
- Trichiniasis.
- Vaccinia.
- Varicella.
- Variola.
- Yellow fever.

2. CONDITIONS ASSOCIATED WITH LOCAL INFLAMMATIONS.

- Acute yellow atrophy of the liver.
- Appendicitis.
- Arthritis.
- Bronchitis, acute.
- Cholangitis.
- Cholecystitis.
- Cystitis.
- Conjunctivitis, acute.
- Endocarditis, acute.
- Endometritis, acute.
- Enteritis.
- Epididymitis.
- Gangrenous conditions: appendix, lung, liver, bowel, mouth, pancreas, etc.
- Gastritis, acute.
- Gastro-enteritis, acute.
- Mastitis, acute.
- Meningitis, septic.
- Nephritis, acute.
- Orchitis.
- Otitis media.
- Ovaritis.
- Pancreatitis.
- Pericarditis.
- Peritonitis.
- Serous effusions into pericardium, peritoneum or joints (non-tubercular).
- Suppuration, in any organ or region of the body.
- Skin diseases: eczema, herpes zoster, pellagra, pemphigus, prurigo.

TOXIC, EXPERIMENTAL AND THERAPEUTICAL LEUCOCYTOSIS

Leucocytosis follows the absorption of certain substances into the blood. Some of these may be toxic in the strict sense of the term, others may not. Such toxic substances as result from bacterial invasion productive of infective or inflammatory processes belong to the former group, but for clinical convenience they are classed separately. The prolonged inhalation of chloroform or ether, poisoning by coal gas and arseniuretted hydrogen, is accompanied by leucocytosis. Blood poisons like phenyl-hydrazin, potassium chlorate and pyridin produce it. It is associated with the toxæmias of renal disease, acute yellow atrophy of the liver, cirrhosis of the liver and jaundice, gout and gastro-intestinal disturbance due to ptomaines.

The subcutaneous injection of a number of substances cause leucocytosis, amongst which are, extracts of spleen, thymus and bone marrow, bacteria and their products, yeast extracts, nuclein and nucleic acid, hemialbumose, pepsine, curare, leech extract, spermine, tuberculin. Many other substances which, when injected, produce aseptic abscess cause leucocytosis, but its degree depends largely upon the amount of irritation the substance produces. Many drugs used therapeutically are known to give rise to a variable amount of leucocytosis. Among these may be mentioned, absinthe, acetic ether, antifebrin, antipyrin, arsenic, camphor, carbolic acid, cinnamon, cinnamate of soda, digitoxin, gentian, morphia, myrrh, oil of anise and fennel, peppermint, phenacetin, pilocarpine (lymphocytosis), piperine, potassium iodide, purgatives such as croton oil, podophyllin, scammony, quinine, salicin, salicylic acid, salicylate of soda, and here may be mentioned transfusion of blood or normal saline solution.

LEUCOCYTOSIS OF MALIGNANT DISEASE AND CACHEXIA

A moderate but variable degree of leucocytosis may be present in cases of malignant disease. It does not occur by any means in every case, and as a rule is more often found in sarcoma than carcinoma and associated with rapidly growing neoplasms with metastases rather than with such as are localized and of slow growth. As a rule it depends upon an increase in polymorphonuclear neutrophiles, but in sarcoma affecting the lymphatic system the gain may be due to lymphocytes. Occasionally, eosinophiles may increase and a few myelocytes be present.

LEUCOPŒNIA

When the number of leucocytes in the peripheral circulation falls below the normal average of the individual, the condition is termed leucopœnia. Some of the physiological factors of leucopœnia have been mentioned with physiological leucocytosis, such as starvation, and its allied conditions, prolonged exposure to cold and a short exposure to heat. In reference to the results upon the leucocyte count of infections, a great deal depends upon the virulence of the process and the degree of resistance offered by the individual. In not a few experimental infections it has been shown that a transient leucopœnia may precede the leucocytosis, and in very acute infectious disease the latter may not take place and the former remain, when the resistance is feeble. In certain infectious diseases, no leucocytosis takes place or even a leucopœnia may be present, which is often of diagnostic value. No leucocytosis occurs in typhoid fever, malaria, influenza, measles, rötheln, mumps, malta fever, leprosy, and tuberculosis when not accompanied by septic infection. In pernicious anæmia the count is low, and in other severe anæmias, especially when the bone-marrow has become aplasic, or anæmias of syphilis and rickets, splenic anæmia, and the severe anæmia associated with gastro-enteritis of children. Strange to say the occurrence of an infection in a case of leukæmia may produce a marked decrease in the number of leucocytes originally present. Certain drugs are known to produce leucopœnia such as ergot, tannic acid, atropine, picrotoxin, agaracin, sulphonal; and the same condition follows the injection of peptone, diastase, and eel serum.

LYMPHOCYTOSIS

An absolute or relative increase in the average number of lymphocytes normal to an individual constitutes a lymphocytosis. In infancy and early childhood the normal number is always greater than in the adult, so much so that a count which would be regarded as normal in infancy might be pathological for an adult.

As a rule lymphocytosis is relative and does not often produce a rise in the total number of leucocytes. When it occurs it is never very great, and as in children a high lymphocyte count is physiological, so in them any increase is always more pronounced than in the adult, and occurs more commonly. In children it is particularly associated with the anæmias of congenital syphilis, scurvy, rickets, gastro-enteritis and tuberculosis. A relative lymphocytosis in the adult is often coincident with a neutrophile decrease and is present in conditions productive of the latter. It occurs in debility, and malnutrition; severe anæmias, chlorosis, pernicious anæmia, typhoid fever, malaria, syphilis and tuberculosis, whooping cough, measles, and scarlet fever. In whooping cough it is very characteristic. In the post-febrile period of measles, in conditions associated with enlarged spleen and malaria, the large mononuclears may be responsible for the increase. Affections of the lymphatic glands often cause lymphocytosis. Certain drugs when injected subcutaneously produce it, notably philocarpin, and amongst others quinine hydrobromate, tuberculin and thyroid extract.

The appearance of great numbers of so-called "lymphocytes" occurs in the blood in lymphatic leukæmia producing an absolute and relative increase, and in the adult such would be the diagnostic feature of this disease.

EOSINOPHILIA

To establish a true eosinophilia there must be an absolute increase in the coarsely granular eosinophile cells above the normal per cubic millimetre of blood. The term is not applicable clinically to a relative increase only. A certain degree of eosinophilia is regarded as physiological in healthy children, and is said to occur in women during menstruation.

With but few exceptions eosinophilia is a blood state of doubtful diagnostic value, as it occurs in varied degree in so many pathological conditions.

Post-febrile.—As has been pointed out before during the period of polymorphonuclear neutrophile leucocytosis the eosinophile cells may be much diminished, and such is the case in most infectious diseases during the height of the fever, with the exception of sacrlet, malarial and rheumatic fevers, in which they may be increased. In the post-febrile period, however, eosinophilia may occur.

Asthma.—In this disease during the paroxysm marked eosinophilia may be present, and the sputum is richly eosinophile. It is said to occur in fibrinous bronchitis.

Skin Diseases.—In many skin diseases such as pemphigus, psoriasis, eczema, dermatitis herpetiformis, prurigo, etc., a pronounced eosinophilia is said to occur. There seems to be more connexion between blood change and the extent of surface involved than the particular form of skin eruption. The investigations of recent date by H. S. French[1] point to the fact that the condition is not so constant in skin diseases as had been previously thought.

Helminthiasis.—In many varieties of helminthiasis eosinophilia is present, particularly in trichiniasis, ankylostomiasis, bothriocephalus, tænia medio-canellata, in ascaris lumbricoides and oxyuris, echinococcus, bilharzia and filaria nocturna.

Myelogenous leukæmia.—In this condition above all others eosinophilia is a constant and striking feature. In the "lymphatic" form, however, there is a diminution in the number of eosinophiles.

Medicinal eosinophilia.—An increase has been found after the injection of camphor, pilocarpine, tuberculin, nuclein, and it occurs in phosphorus poisoning.

Other causes.—It may occur to a moderate degree in malignant disease, especially if bones or marrow be implicated, in anæmic and purpuric states, in severe ovarian disease, gonorrhœa (the exudate often contains large numbers of eosinophile cells), gout, after splenectomy and in certain forms of splenomegaly. Its association with lymphocytosis in children may be indicative of congenital syphilis.

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[1] *Brit. Med. Journ.*, April 30, 1902.

MYELOCYTOSIS OR MYELÆMIA

The myelocyte proper, whether neutrophile or eosinophile, in its mononuclear form, is a cell foreign to normal blood, its natural habitat being the bone-marrow. If, however, the transitional variety is to be regarded as a phase between the neutrophile myelocyte and the common neutrophile polymorphonuclear of normal blood, then one must acknowledge that in this stage it appears normally in small numbers. In conditions of profound leucocytosis, neutrophile myelocytes may appear, but they are few in number. They appear in conditions associated with bone-marrow disturbance such as grave anæmia, profound chlorosis, pernicious anæmia, rickets, osteomalacia, osteomyelitis ; also in acute infectious diseases and sepsis, typhoid fever especially in children, diphtheria, malaria, uræmia, diabetic coma, carbon monoxide poisoning, puerperal mania, exophthalmic goitre, general paralysis of the insane, syphilis and malignant disease. Perhaps with the exception of malaria, typhoid fever and diphtheria, their occurrence in the above-mentioned conditions is never marked. They are observed in Hodgkin's disease and lymphatic leukæmia. There are three diseases in which myelocytes are notably present ; in myelogenous leukæmia they are the characteristic feature, occurring both as neutrophile myeloctyes and mononuclear eosinophile cells. In chloroma they are present in large numbers, and also in the peculiar blood condition known as leukanæmia. They also occur in lymphoid leukæmia and Hodgkin's disease, but not to any marked extent.

Leucoblastæmia.—While considering the occurrence of myelocytes in the blood, I would point out again the opinion, held personally and by others, that the cell of acute or lymphoid leukæmia is not a lymphocyte proper, but a myelocyte antecedent. Assuming the correctness of this view, it will follow that myelocytes in the early stage of their life history are present in the blood in acute leukæmia, and as such give the pathognomonic appearance to the blood in this disease. They also occur in chloroma, leukanæmia, and the mixed forms of leukæmia. In myelogenous leukæmia, as before stated, I have seen the blood picture change from the ordinary type to that of acute leukæmia by the marked increase in the number of these leucoblastic cells.

In some cases of leukæmia myeloplaxes or giant myelocytes may be found in the blood.

BASOPHILIA

An increase of basophile cells in the blood stream is a rare occurrence, and they are never abundant. They have been found in chlorosis, various diseases of the skin, the late stage of asiatic cholera, in severe cachexia due to carcinoma, in gonorrhœa and acute septic disease of bones, in splenic anæmia, mycosis fungoides, Hanot's cirrhosis of the liver, and in one case I found many basophile cells in the exudate from vesicles due to acute iodism.

In myelogenous leukæmia they are present in considerable numbers, and vary both in size of cell and the intensity of granulation.

The clinical significance of basophile leucocytosis has not been determined.

THE NATURE OF LEUCOCYTOSIS

Physiological leucocytosis remains yet to be explained satisfactorily, probably the causative factors act in a similar way to those which produce pathological leucocytosis. In the latter it is self evident that some factor of special import is at work in order to produce the rapid and abundant influx of leucocytes above the normal into the blood stream. In inflammation and local suppuration enormous numbers of leucocytes come by the blood stream to form pus.

The fact that a comparatively small amount of pus would represent all the leucocytes of normal blood present at any one time, a suppurative process would rapidly exhaust the blood of its leucocytes unless a continuous supply be kept up. This continuous influx must come from somewhere, and the two main sources of it are the bone-marrow and lymphadenoid tissues throughout the body. Foremost of these is the bone-marrow, for it is doubtful how far the lymphatic tissues share in active leucocytic emigration in view of the feeble motility of its cells. Leucocytosis is mainly dependant for its cell constituents upon the motility of the cells, and so the more active polymorphonuclear neutrophiles and the eosinophile cells are the varieties which appear in the blood stream. In inflammatory and infective processes the polymorphonuclear neutrophiles are the cells which predominate, but there are certain conditions in which the eosinophiles are most in evidence.

It would seem that in some peculiar way the marrow, which is the manufactory and storehouse of leucocytes is roused into greater activity, and large supplies of leucocytes are produced which pass into the blood stream. This activity is brought about by substances present in the circulating blood which stimulate the marrow and to which it responds in a definite and specific manner.

It is known that changes in the composition of a fluid produce definite effects upon the motility of living organisms. In certain ferns Pfeffer[1] discovered that the malic acid present in the female sperm cells exercised an attractive influence upon the spermatazoids, and that strong solutions of the acid repelled them. To this peculiar phenomenon he gave the name chemotaxis; attraction being due to positive, and repulsion to negative chemotaxis. Stahl[2] while experimenting with plasmodia found that certain substances attracted, others repelled them, and that the movements took place irrespective of position. The phenomenon of chemotaxis is the basis of leucocytosis, and was first demonstrated by Leber [1], who extracted from staphylococci a crystalline substance—*phlogosin*—which exercised a positive chemotaxis towards leucocytes, and he showed that they migrated to the seat of infection by bacteria, and that the attraction was due to bacterial products. Other chemical substances, however, which are not the outcome of bacterial metabolism or disintegration, may act in the same way, and the products of tissue and other cell disintegration are chemotactic. Some are positive, others negative.

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[1] *Untersuch. aus dem. Bot. Institut in Tübingen*, vols. i. and ii. 1881–1888.

[2] *Botanische Zeitung*, 1884.

Leucocytosis is due, then, to a positive chemotaxis towards leucocytes, exercised through absorption into the blood stream of substances arising from the metabolism or disintegration of pyogenic organisms, resulting in a marrow response in the form of increased manufacture and emigration of motile cells, and in special conditions even myelocytes and leucoblasts. That the latter have some degree of motility, though slight, accounts for their presence in acute leukæmia, and in one case which came under my notice, with a pleural exudate, the cells in the pleuritic fluid were practically all of this variety.

The effect upon the bone-marrow is shown morphologically by increased proliferation of its cell elements, and a coincident increase in the tissue as a whole, so that areas not usually the seat of red marrow become encroached upon. Chemotaxis is not regarded as applicable to the production of lymphocytosis, the motility of these cells being minimal. Their appearance in the blood may be regarded as a passive emigration, and in local accumulations the increase may arise by proliferation in situ.

The same substance is not positively chemotactic to all leucocytes alike. What may attract one variety may be indifferent or even negative to another. As a rule in experimental leucocytosis, a primary and transient leucopœnia is followed by a polymorphonuclear increase, then an increase of lymphocytes, and finally the eosinophiles which were absent or diminished during the initial leucocytosis reappear and may be increased for a time.

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[1] *Fortschritte der Med.*, 1888, vol. vi, p. 460.

Leucocytosis, chemotaxis, and phagocytosis are all intimately related, and phagocytosis may be regarded as a further expression of chemotaxis, so that the bacteria or other bodies which give rise to the chemotactic substance are themselves engulfed by the leucocytes. This, however, does not depend solely upon a reaction between leucocytes and bacteria, for certain substances are present in the serum which so act upon the bacteria as to favour their being taken up by the leucocytes. Sir A. E. Wright and Douglas, who first demonstrated this relation of serum to phagocytosis, have shown, and the fact has been corroborated by others, that, if leucocytes be washed free from serum and incubated with certain organisms, little or no phagocytosis takes place. If, however, normal serum be added phagocytosis occurs, and is due to the presence in the serum of substances which they term "*opsonins.*" The opsonin acts upon the bacteria rather than the leucocytes, but the nature of the action is unknown. The opsonin may be destroyed by a temperature of 60° C. in ten minutes. It is supposed that negative chemotaxis depends upon substances which are inimical to or destroy the opsonins.

Although phagocytosis depends largely upon the action of extracellular substances on bacteria, the fact certainly remains that leucocytes do take up living bacteria, which may even multiply within them, but they do not engulf very virulent organisms.

How far the tissue cells enter into the rôle of phagocytosis is not fully determined, they are phagocytic to a certain extent, but take a much lower position than the leucocytes, and Ruediger [1] has shown that emulsion of marrow destroys streptococci, but other organs do not. The bone marrow must be regarded as a protective tissue, and I consider that in it we have a specialized organ for the purpose of protecting the body within from the unseen enemies which invade it from without. In this rôle it is as special an organ as any other, as important in a sense as the central nervous system, and like it, for its own safety, is situate within dense and strong coverings, the bones.

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[1] *Journ. Amer. Med. Assoc.*, 1905, vol. xliv, p. 198.

CHAPTER IX

ANÆMIA AND ERYTHRÆMIA—ANÆMIA IN GENERAL—ACUTE POST-HÆMORRHAGIC ANÆMIA—SIMPLE CHRONIC SECONDARY ANÆMIA—PRIMARY ANÆMIA—CHLOROSIS—PERNICIOUS ANÆMIA — TABLE — APLASTIC ANÆMIA—SPLENIC ANÆMIA—ERYTHRÆMIA.

ANÆMIA

THE term anæmia is used to indicate loss of blood as a whole (oligæmia), or any one of its most important constituents, erythrocytes, hæmoglobin, albumin, with or without changes in the total volume of blood. The term has for long been associated with pallor of the skin and mucous membranes, but pallor alone is not always indicative of blood change. Many people whose appearance would lead one to regard them as anæmic show no distinctive abnormal blood state. Pallor is not incompatible with polycythæmia and superabundant hæmoglobin, while the converse is equally true that a good colour may be associated with anæmia. Only by an examination of the blood can the condition be recognized with certainty. The two chief alterations in the blood which are clinically associated with anæmia are erythrocyte or hæmoglobin loss, or both. These are of paramount importance but they are almost invariably accompanied by qualitative or quantitative alterations in other constituents. Morphological alterations in the cellular elements is a more or less constant accompaniment of the anæmic state. All factors causative of anæmia operate by denutrition of the blood in some or other way. This impoverishment may take place by direct loss, by continued drain from the blood of its more important constituents, or by lack of supply to the organism of materials necessary for nutrition. Taking the last first, deficiency of food necessary for nutrition and growth is a well recognized cause of anæmia, especially in young people. Nutriment, may, however, be sufficient or abundant, but rendered useless by perversion of the natural processes which prepare it for assimilation, or to mechanical causes which obstruct its admission or transmission through the natural receptive channels. The very causes which produce anæmia by perverted digestion or assimilation are intensified by the anæmia itself, so that a vicious cycle is determined which tends to prolong and aggravate the condition. The blood change is rarely due to a single cause, there is generally a multiplicity of them. Not only lack of nutriment, but unhygienic environment or occupation, mental and bodily exhaustion all tend the same way. In reference to blood loss, or drain, hæmorrhage as a cause is self-evident, it is rapidly recovered from when sudden and profuse within limits; but continued or intermittent small hæmorrhages from the mucous surfaces may produce an anæmic state, which may, from lack of recuperative power of the hæmogenetic tissues, become "progressive," and fatal. It is quite conceivable that here, also, a vicious cycle is established, so that the impoverished state of the blood itself increases the tendency to hæmorrhage, which originally produced it. Apart from direct loss due to hæmorrhage, many other factors are responsible for anæmic states by blood drain or destruction. In this category are included chronic suppuration, intestinal discharges, chronic renal disease, fevers, infectious processes, intoxications, the entrance of toxic materials such as lead into the system, and parasitic diseases, e.g. malaria. The anæmia of malignant disease may, perhaps, be caused by a toxic substance derived from the neoplasm, or the effect of the growth upon digestion and assimilation. Thus more profound anæmia is an almost constant concomitant of neoplasms of the digestive tract than in other situations. In the latter it is never so marked unless associated with hæmorrhage or chronic discharge.

The above short resumé of the causes of anæmia point to it as a result of interference with hæmogenesis, or direct hæmolysis, and as such is regarded as "symptomatic" or "secondary." It may rightly be assumed that all anæmias are "secondary." But there are some forms for which, in the light of our present knowledge, no adequate or definite cause can be assigned; and these have to be regarded as depending upon deficient hæmogenesis on the one hand, or excessive hæmolysis on the other, the ultimate nature of which remains obscure. Anæmias of this class are designated "essential" or "primary," and of these two are recognized clinical entities, with characteristics peculiar to them and which differentiate them one from the other, and from the "secondary anæmias." I allude to *chlorosis* and *progressive pernicious anæmia*. They contrast strongly with one another.

Chlorosis is regarded as due to defective hæmogenesis. It is almost entirely confined to the female sex; rarely, if ever, found in its true form in males. Occurring, as it does, about the age of puberty, it has a close relation to the evolution of the sexual organs. Recurrences are common, may extend into the third decade of life, but the condition rarely of itself proves fatal. There is evidence of hereditary tendency. Although the general causes of anæmia may play a part in the determination of this condition, it is doubtful if they can produce it exactly in an individual free from a chlorotic predisposition. Many theories have been promulgated to explain its etiology. Virchow regarded it as part of a general hypoplasia of the vascular system and genitalia, but such hypoplasia occurs in individuals who never evince any sign of chlorosis. The special feature of the disease, lack of hæmoglobin, has given rise to attempts to explain its etiology by some perversion of the metabolism of iron within the body. By the iron being stored up in the internal organs in females at the time of puberty, in anticipation of conception and gestation, and for the purpose of supply to the fœtus during the latter period, it is supposed a lack of hæmoglobin occurs in the blood. The association of disorders of digestion and assimilation with chlorosis has led to the observance of these as causative factors leading to a deficiency of iron in the blood. Among the alimentary disturbances have been included, dilatation of the stomach, gastroptosis ("corset chlorosis"), alteration of hæmoglobin formation in the mucous membrane of the intestine, constipation and auto-intoxication from the alimentary canal.

The blood state in chlorosis has been regarded as symptomatic of a peculiar neurosis. That it may be the result of repeated small hæmorrhages from the alimentary canal which have escaped notice presents points for credence in view of the serious hæmorrhages from the stomach which occasionally take place in this disease, and for which no gross lesion such as ulceration is found.

It is doubtful if, as has been suggested, it can be regarded as a disease due to some specific infection. In the light of recent advances in our knowledge of the "internal secretions" of glandular organs, there is reason to entertain the possibility of chlorosis depending upon a lack of stimulation of the hæmogenetic tissues by the sexual organs, and that some close relationship exists between the periodic loss of blood by menstruation and a possible stimulus proceeding from the sexual organs for the purpose of hæmogenesis to replace loss. Should any functional weakness of the hæmogenetic tissues, either congenital or acquired, exist, no response to stimulation from the reproductive organs would be forthcoming; or, on the other hand, if the stimulus be wanting, the result would be the same, namely chlorosis. Deficient hæmogenesis, whether congenital or acquired, seems to be the real foundation of the chlorotic state. The fact that amenorrhœa is so often associated with the condition points to an effort on the part of nature to prevent excessive loss, which if it persisted along with deficient hæmogenesis would sooner or later lead to disaster. In the female some special reserve function of the hæmogenetic organs may exist to balance the repeated loss of blood at the menstrual period, which goes on for so many years, and when in chorosis, this reserve is called upon to take the place of ordinary hæmogenesis common to male and female, the menstrual process is kept in abeyance.

When we come to consider "primary progressive pernicious anæmia," we find it far removed from chlorosis in many characteristics. It has no necessary association with sex or development, it is common to both sexes. It does not depend upon any of the usual causes provocative of anæmia, and presents a characteristic blood picture quite different from chlorosis and the forms of simple secondary anæmia usually met with. At one period regarded as a result of defective or perverted hæmogenesis, it has more recently been accounted a condition resulting from excessive hæmolysis, and subsequent exhaustion of the hæmogenetic tissues. We are not sufficiently acquainted with the factors which constitute senility to be able to allot to any organ a definite time limit to its power of functionating. We note exceptions to this in the organs associated with procreation. What the allotted period of action of the bone marrow, for example, may be is unknown, but there is reason to believe that it may become prematurely old, either from original or acquired deficiency in its power to carry on its function, or congenital insufficiency. A tendency to rapid exhaustion, and inability to respond to the calls made upon it, may determine the fatal character of the anæmia which is termed "pernicious," or congenital defect may determine its premature senility. Thus one meets with instances of pernicious anæmia in which heredity must have played a part. I have met with it in a mother and her two sons, all of whom died from the disease. Apart from any lack of hæmogenesis, the erythrocytes formed may be more susceptible and less resistant to influences which tend to hæmolysis. There may be several distinct clinical forms of the disease associated with different etiological factors; some have been associated with pregnancy and parturition, with profuse hæmorrhage, with atrophic changes in the alimentary mucous membrane, or with the presence of parasites. It has been supposed that the causes productive of ordinary anæmia may go on to the determination of the fatal pernicious type. The effects of toxic substances such as toluylendiamin, nitro-benzole, etc., which produce blood states practically indistinguishable from pernicious anæmia; the effects of the hæmolytic poison of Bothriocephalus latus; the evidences of hæmolysis manifested by the increased amounts of iron in the liver and other organs, the hæmoglobinæmia and icterus, all point to the condition being one of protracted hæmolysis eventually leading to hæmogenetic exhaustion. Some of these hæmolytic agents we know, others remain conjectural. Where and how they arise is not absolutely determined. Some are referred to infections of unknown nature occurring in the mouth or intestinal tract producing materials toxic to the blood or bone marrow. That it is the result of septic processes has been strongly advocated by Dr. W. Hunter [1] who lays particular stress upon oral and intestinal sepsis. It is certainly very striking how often oral sepsis is present; and I have seen this anæmia follow suppuration within the antrum. Sooner or later it may happen that the problems of this disease will be elucidated and the definite causes discovered, but up to the present there still remain cases the etiology of which is absolutely unknown, and which conform to Addison's description of the disease as "general anæmia occurring without any discoverable cause whatever, cases in which there had been no previous loss of blood, no exhausting diarrhœa, no chlorosis, no purpura, no renal, splenic, miasmatic, glandular, strumous, or malignant disease." Although it would seem that, with advanced knowledge in the future all anæmias will be proved to be "secondary," those cases for which no definite etiological factor can be discovered until then must be regarded as "primary" or "essential."

ACUTE POST-HÆMORRHAGIC ANÆMIA

As the name implies, this form of anæmia is associated with rapid and more or less profuse loss of blood, such as may follow a wound of a large vessel with bleeding externally or into one of the body cavities, or from spontaneous hæmorrhage such as is met with in abortion, the puerperium, ruptured tubal pregnancy, uterine tumours, ulceration of the mucous membrane in any part of the alimentary tract, either simple or malignant, from typhoid and similar ulceration, in cases of pulmonary tuberculosis with profuse hæmoptysis, from the sac of a ruptured aneurysm, from varicose veins of the œsophagus, hæmorrhoids or subcutaneous varicose veins, from the nasal mucous membrane, hæmorrhage into the pancreas, and the hæmorrhagic group of blood diseases as hæmophilia.

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[1] *Pernic. Anæmia*, London, 1901; *Lancet*, January 27, February 3, 10, 1900. *Oral Sepsis as a Cause of Disease*, London, 1901; *Index of Treatment* (Hutchison & Collier), 1908, 3rd edit., p. 618.

Symptoms

Closely associated in time with a profuse hæmorrhage are symptoms indicative of collapse, pallor of the skin, and mucous membranes, with weakness, vertigo, feelings of coldness and fainting. The weakness may be extreme. The voice becomes feeble and tremulous, flashes of light appear before the eyes, tinnitus aurium and parosmia occur, and a sense of great anxiety. The body is covered with perspiration and the urine may be increased. The pulse is frequent, soft, changeable in rhythm and tension. Nausea and vomiting occur, with intermittent attacks of syncope. As the weakness increases, somnolence, aphasia, paraphasia and delirium may be present, with extreme dyspnœa. At a later stage the perspirations cease, the skin dries, the voice is lost, the eye becomes lustreless, contractions of groups of muscles or general convulsions may come on, the pulse and respirations are feeble and slow, the body temperature falls, and death ensues if the hæmorrhage does not cease, or cannot be controlled.

Should the hæmorrhage cease or be permanently controlled the symptoms which follow will depend upon the amount of blood which has been lost. Profound anæmia in severe cases may last for some time, and with it a series of symptoms associated with the various organs. The skin and mucous membranes remain pallid, and there is generally some œdema of the ankles not necessarily dependent upon kidney changes, although from the latter and blood change albumin and hyaline casts may appear in the urine.

The effects of the blood loss upon the heart and vessels is productive of a degree of fatty degeneration, responsible on the one hand for irregularity of rhythm, the onset of palpitation from slight exertion and general cardiac weakness, sometimes with syncopal attacks, or even collapse. Hæmic murmurs are heard at the valve areas and in the large veins. Partly from the fatty degeneration of the vessels and the altered blood-state, spontaneous hæmorrhages may take place from the mucous membranes, the gums, nose, uterus, conjunctiva, etc.

Dyspnœa occurs on slight exertion. Loss of appetite, with impairment of digestion is a usual symptom, but in some instances the appetite may be voracious. A tendency to headache and neuralgias are the principal nervous symptoms, but associated with fatigue, transitory aphasia and thickened speech are not uncommon. Hæmorrhage into the optic nerve may cause blindness or dimness of vision. Transient amaurosis without ophthalmoscopic findings, may occur, but hæmorrhages sometimes take place into the vitreous and retina, with neuro-retinitis, optic neuritis or even atrophy.

The progress is generally favourable if the anæmia be uncomplicated, and recovery gradually takes place, with disappearance of symptoms. The prospect of recovery is influenced by age and nutrition prior to the hæmorrhage. It is less favourable in the young and aged than at the more robust periods of life. The presence of previous disease has an adverse influence on the result. For some time the health may remain unsatisfactory even after the blood has been restored to normal, or, as occurs in some cases, the peripheral blood count may overreach it.

The Blood in Acute Post-Hæmorrhagic Anæmia

If the hæmorrhage be profuse a loss in the volume of blood takes place, constituting a true oligæmia, but if it be only moderate the loss is made up so rapidly that a definite oligæmia may not be noticeable. The reduction in volume is compensated by absorption of the tissue fluids, resulting in an increase of the watery elements or hydræmia, and which is more marked the greater the hæmorrhage. The specific gravity of the blood falls, but the dried residue of serum remains practically the same, so that the increase of fluid elements is not due to water alone. There is a diminution in the number of erythrocytes and the amount of hæmoglobin. This does not reach its maximum until a few days after the hæmorrhage, is gradual, relative, due to absorption of fluid, and more marked and prolonged the greater the hæmorrhage. Owing to the loss of corpuscles and proteids the " total alkalinity " is lowered, from lack of " fixed alkali." The blood picture may show nothing abnormal, or it may reveal morphological alterations. Normoblasts appear, they may be few or abundant, and a " blood crisis " in which they appear in large numbers may occur. After such an influx the rapidity of erythrocyte regeneration is augmented. Megaloblasts are rarely present. Many of the erythrocytes themselves appear swollen and increased in bulk after a severe hæmorrhage, so that their percentage volume, as indicated by the hæmatocrit, is greater. The swelling of the erythrocytes is due to the hydræmic state of the blood, for the absorption of water is more rapid than the salts, so that the plasma becomes hypotonic relative to the cells. If the isotonic strength of the plasma fall below 0·8 per cent. NaCl solution swelling will take place, and even hæmolysis happen if the fall be below 0·46 per cent. The diameter of the erythrocytes may vary, and mycrocytes and poikilocytes appear. Deficiency of colour need not necessarily follow in all the cells, but may be marked in some, and reduces their reaction to stains. Polychromatophilia occurs early and may continue until recovery has taken place. First a relative and then an absolute leucocytosis follows the hæmorrhage. It is usually of the polymorphonuclear neutrophile type, and gradually returns to normal as blood regeneration proceeds. Many of the cells may lack granulation. There may be a transient lymphocytosis in some cases, and in rare instances myeclocytes appear. The leucocytosis is accounted for partly by their adhesion to the vessel walls preventing loss, partly to an influx of lymphocytes from the lymphatic tissues or to a definite stimulation of the bone marrow. There is a numerical increase of blood platelets and a greater power of coagulation, so that the coagulation time may be considerably reduced. The effect of the loss of blood will depend upon the age, sex and nutrition of the individual, is more pronounced in the very young and old, or in those who have been previously debilitated from other causes. The amount of hæmorrhage necessary to fatality varies, but recovery may follow even when the erythrocytes fall to 11 per cent. of the normal. Regeneration depends upon the initial severity of the hæmorrhage, the age and nutrition of the individual, and is less likely to occur in early infancy or old age. Gradually the normoblasts disappear, the erythrocytes recover their shape and size, the hæmoglobin assumes proper proportions, although it does not increase at the same rate as the corpuscles, so that the colour index will be low at first, but gradually rise. Polychromatophilia disappears, the leucocytosis falls, and in about three weeks to a month the blood will have recovered itself even after a hæmorrhage sufficient to reduce the hæmoglobin to 25 per cent.

Treatment

The arrest of traumatic hæmorrhage comes within the domain of surgery. In cases of spontaneous hæmorrhage, absolute rest, both physical and mental, is essential, and to assure this the administration of opium or one of its preparations holds a premier place. It is not within the scope of this work to describe in detail the treatment of hæmorrhage from special localities, but many drugs have been advocated as hæmostatics, such as perchloride of iron, acetate of lead, gallic and tannic acids, stypticin, hamamelis, salts of calcium, preparations of ergot, adrenalin chloride, gelatin in solution by mouth or rectum, the inhalation of amyl nitrite, nitro-glycerine and nitrite of soda, but there is a marked diversity of opinion as to their individual utility. The local action of adrenalin upon mucous membrane as a hæmostatic is unique.

After the hæmorrhage has ceased, absolute rest is demanded for some considerable time, not only from the fear of a recurrence, but to prevent the onset of syncopal attacks. If syncope tend to recur the injection of small quantities of warm water may be found useful ; in some cases when collapse is threatening or present, the fear of immediate dissolution has to be met by more stimulative measures. I could never understand the rationale of the application of cold to the body surface, especially in cases of internal hæmorrhage from mucous membranes—warmth is a more reasonable measure. In severe cases auto-transfusion by bandaging the extremities and elevation of them may be resorted to for the purpose of cerebral and cardiac supply. Transfusion of sterilized normal saline solution at the body temperature into a vein may be demanded, but must be used with caution ; injection of it into the areolar tissues subcutaneously is valuable, and allows gradual absorption, and rectal injection is perhaps equally beneficial. The transfusion into a vein of defibrinated human blood has its advocates, but it is difficult to obtain ; blood from the lower animals is not to be recommended and either procedure is not devoid of danger. The injection of defibrinated blood into the areolar spaces is painful, and presents no special claims above normal saline solution, for apart from the fact that it provides a certain amount of fluid, it has no effect towards hastening recovery. When hæmorrhage takes place into a serous cavity, in which case the blood is retained, and the same holds good in hæmorrhage into the cellular tissues (equivalent to the artificial injection of defibrinated blood), there is no difference in blood restoration than when the loss is external and absolute.

After the earlier effects of the blood loss have passed off, and the anæmic condition has to be treated in the hope of aiding blood restoration and with it convalescence, prolonged rest is essential because of the tendency to syncope, and the distressing dyspnœa which are associated with exertion.

The thirst which is complained of may be best relieved by small quantities of fluid. The diet should be easily assimilable ; at first liquid, then gradually reverting to solid food rich in iron. For this purpose small quantities of underdone or even raw meat are most beneficial.

The symptoms of dyspepsia may be treated on general principles, and attention must be directed to the relief of neuralgias. The administration of iron is not so effective in this as in other anæmic conditions. If administered in inorganic form, the preparation chosen should be a mild one with a minimum tendency to produce gastric irritation. Organic preparations are useful and easily absorbed. The benefit derived from iron rests not only on its absorption as a metal but by stimulation of the bone marrow towards blood formation. It should be administered with or shortly after food; never on an empty stomach. Preparations of hæmoglobin may be useful and are easily absorbed.

SIMPLE CHRONIC SECONDARY ANÆMIA

In contrast to the preceding form of anæmia, this is generally associated, not with an acute profuse hæmorrhage, but with repeated small hæmorrhages which gradually reduce the blood or its constituents. The general causative factors in this condition have been already mentioned in the article on anæmia in general. How far they individually may be responsible for the anæmic state it is difficult to decide. Lack of fresh air and food proper for blood production and nourishment are potent in their effects. The quantity of food is quite unimportant as compared to its blood-sustaining qualities, and it is those individuals who are deprived of food rich in iron that suffer most; in children, for instance, who are fed on milk beyond the proper limits of time anæmia is common, unless iron-rich food is given also. The poor, to whom a regular meat diet is beyond their means, soon show signs of blood deterioration. It must be admitted, however, that prolonged insufficiency of food would include a poverty of blood-forming materials, and anæmia follow; but rapid withdrawal of food, even completely, for a limited period, as in professional fasters, does not produce oligocythæmia or oligochromemia. Light is not an essential factor for blood formation or sustenance. It seems that it has no direct effect.

Among other causes of chronic anæmia are the drain made upon the blood by prolonged suppuration, albuminuria and toxic conditions associated with it. The toxæmia accompanying pyrexias, syphilis, malaria, neoplasms, of a malignant character, and the introduction of metallic poisons, such as lead, arsenic, benzine derivatives; the presence of helminthes in the blood, tissues or alimentary canal, of which filaria sanguinis hominis, ankylostomum duodenale, which acts both by mechanical withdrawal of blood, and the effects of toxic materials in the head of the worm which retard coagulation, and bothriocephalus latus, are the worst.

Symptoms

These differ slightly, if at all, from the later stages following acute hæmorrhage. The skin may assume a sallow, rather than a white colour. In severe cases there is well marked muscular weakness, and an easily fatigued nervous system. There is a tendency to œdema, and in prolonged cases to spontaneous hæmorrhages from the mucous membranes, and even into the central nervous system, the retinæ or serous membranes.

Digestion is disturbed, the appetite capricious, pain after food may simulate ulcer of the stomach. The motor mechanism, secretion and absorption power of the stomach are but slightly impaired, but constipation may occur.

The question is unsettled as to how far the anæmia produces the digestive disturbance, or the latter the former, or that their relation is one of mutual reaction.

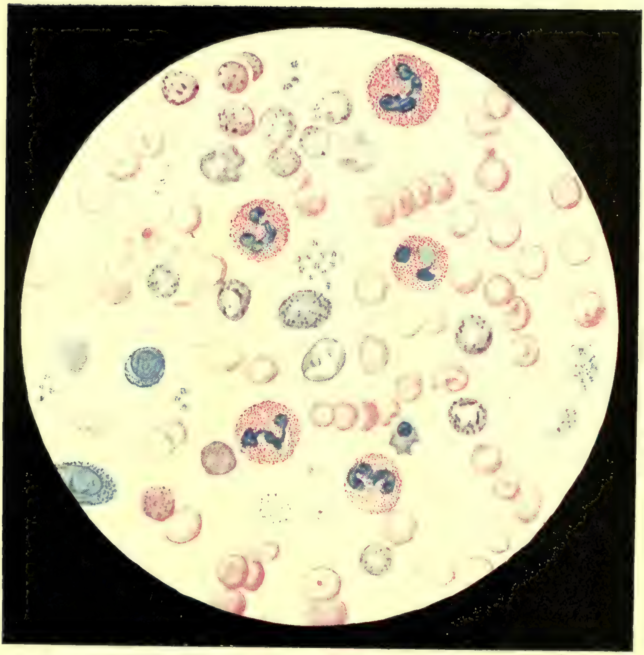
The Blood in Simple Chronic Secondary Anæmia

The blood after chronic and repeated loss or impoverishment secondary to disease may not show changes much different from those which are present during certain transient periods following acute hæmorrhage; on the other hand, it may resemble chlorosis or even primary pernicious anæmia. The total volume, which in acute hæmorrhage is immediately decreased and so rapidly regained, may not be diminished in chronic anæmia; in fact, it is generally increased. The drop of blood drawn from the peripheral circulation usually appears lighter in colour than normal, and stains fabrics less deeply; it may appear thinner in consistence, flow tardily or easily, and the cellular elements collect together to move about in the plasma, as is sometimes seen with certain water colours when mixed with water on a paint palette. The reduction of hæmoglobin is variable, it may fall to a very low percentage. The red corpuscles are numerically decreased, and the fall may coincide in amount with the hæmoglobin, the two going together, but more commonly the hæmoglobin loss is disproportionate to that of erythrocytes, being greater, so that the colour index is low, resembling chlorosis. The blood contains excess of water, chlorine and sodium, while the potassium salts and phosphates are diminished. The percentage of iron in the blood is diminished proportionately to the degree of the anæmia. The alteration in the amount of dried substance indicates the degree rather than the nature of the anæmia. The specific gravity is diminished proportionately to the hæmoglobin. The alkalinity varies, in some cases it has been found diminished, in others normal or increased.

The coagulation time is, as a rule, shortened, and the separation of clot and serum takes place in a shorter period than normal.

The morphology of the erythrocytes is altered, they are diminished in size, never increased. They vary in their affinity for stains, some appear normal, others stain faintly, and the colour, in advanced conditions, disappears entirely from the centre, so that only a peripheral ring remains (pessary forms). The pallor and irregularity of staining may be observed in the majority of the erythrocytes, especially in anæmia of high degree. Polychromatophilia and punctate basophilia may be present, but are in no way related to the amount of colouring matter. Not only are the erythrocytes below normal in size, but poikilo-

PLATE XII.



cytosis may be marked, but the altered cells are small. These changes in contour depend largely upon the loss of hæmoglobin. Normoblasts occur, but their numbers vary from day to day, and are no reliable indication of the severity of the anæmia. Occasionally a megaloblast may be found in severe cases.

A leucocytosis is generally present, and when polymorphonuclear in type, the lymphocytes and eosinophiles are relatively diminished. If the proportion of leucocytes and erythrocytes remain normal, the eosinophile count may be high. In severe cases the lymphocytes may be increased and myelocytes occasionally be found. Basophile cells do not increase. In some forms of secondary anæmia the leucocyte count may be normal or reveal a leucopœnia, in association with a relative lymphocytosis in severe cases.

The leucocytosis is regarded as resulting from a stimulation of the bone marrow, and depends upon the cause of the anæmia.

Blood platelets are generally increased in numbers, but the alteration is by no means constant.

Treatment

In reference to the *treatment*, a study of causal factors will give the first intimation of rational methods to be exercised in hope of cure. Either the individual must be removed from the cause, or the cause from the individual; this is an essential procedure, prior to all other therapeutic measures. If the true etiology of the condition be discovered and recognized, treatment is rendered more accurate and with promise of good result. Rest, fresh air, genial and health-giving surroundings, attention to diet and proper food, are of paramount importance. Attention to the digestive functions and the administration of one of the easily assimilable preparations of iron, either inorganic or organic, is desirable. Special cases will require special treatment, according to the etiological factor, but it is beyond the scope of the present work to discuss them individually here.

PRIMARY ANÆMIA

CHLOROSIS

Reference has been made to the etiology of this condition in the introductory remarks on anæmia, and they need not be repeated here. Although considered as a primary anæmia, with special peculiarities which distinguish it in many ways from simple chronic secondary anæmia, it is regarded by some that certain physiological and pathological factors so often associated with it, and which operate in its causation, are sufficiently definite to explain its origin, and remove it from the category of a primary or essential anæmia, and establish it as a true secondary anæmia. Of the pathological factors the principal one is sepsis, oral, gastric and intestinal, and their presence is accounted sufficiently productive of the disease in varying degrees of severity, and are potent in the resistance to treatment which obtains in cases associated with them. Another factor is lack of food poor in iron, either due to inability to obtain it, or what is quite common the perverted appetite which leads to its elimination from the dietary.

Symptoms

The *symptoms* of chlorosis are very characteristic. It may be that the earliest complaint is of a feeling of tiredness and exhaustion, with dyspepsia, dyspnœa on exertion and palpitation. Quite early, but in varying degree, pallor of the mucous membranes is noticeable. The complexion has a creamy appearance, with a pink flushed cheek, of which the chlorotic girl is generally proud. In severe cases the complexion may be sallow or even of a greenish tinge, the latter less often seen now than it was years ago. This tinge of the skin is more noticeable in the brunette than the blonde. A certain amount of pigmentation may be present about the neighbourhood of the joints.

The degree of pallor and general muscular weakness do not necessarily go together; some individuals with but slight chlorosis complain much more of weakness than others in whom pallor is pronounced. The weakness is generally most marked in the morning and towards evening passes off, so that vivacity takes the place of lassitude.

In spite of the anæmia there is little or no emaciation, and though in some it may be present, in many profound chlorotics the body remains well covered, and they may even gain in weight. A certain amount of œdema about the ankles is not uncommon, but not associated with albuminuria. Digestive disturbances are common, and complaint is made of pain after taking food, flatulence, eructations, and heart-burn. The appetite is perverted, with dislikes for certain articles of diet especially meat, and inordinate desires for others. Loss of appetite may be slight or so severe as to reach complete anorexia. Constipation is present in the majority of cases, but is not a constant feature and may vary much in degree.

Chlorosis is not uncommonly associated with gastroptosis, enteroptosis and movable right kidney.

The circulatory system is disturbed. Palpitation may be distressing, and with it dyspnœa; both easily produced on exertion. The pulse is soft and easily altered in rhythm and tension, so that it may be dicrotic. Pulsation may be visible in the veins. The heart may be dilated as evidenced by percussion, and hæmic murmurs be audible at any area, but particularly the pulmonary. The loudness of the murmurs does not coincide with the intensity of the chlorosis, for in some mildly chlorotic, loud murmurs may be heard; in others markedly chlorotic the murmurs may be but slight. The *brûit-de-diable* in the veins of the neck may be loud or faint, and bears no definite relation with the intensity of the chlorosis, although it and other venous murmurs, the orbital and cephalic, are generally heard best in those in whom chlorosis is profound.

With the weakness of the circulatory system is associated the coldness of the hands and feet, about which so many chlorotics complain. A tendency to syncope or vertigo is often met with, not in the recumbent posture, but after standing for any length of time, and this more often while standing still than when in the upright posture and moving about.

Chlorotics are regarded as liable to venous thrombosis, particularly in the femoral veins, and even in the cerebral sinuses, but I don't think it can be accepted as a frequent complication. Its presence may give rise to pulmonary embolism, and the possibility of this danger should not be forgotten. Among nervous symptoms mental disturbance may be evidenced by dulled cerebration; or, on the other hand, by mental activity and a peculiarity of disposition, whimsical, or what is frequent, extreme wilfulness and obstinacy, more often seen in the well-to-do. Headache is common, and chiefly complained of in the morning on assuming the upright posture; it is relieved by recumbency, may pass off as the day advances, but in severe chlorotics it may be a constant symptom present even while lying down. Ocular symptoms occur in the nature of floating spectra, sudden but transient attacks of dimness of vision, or even complete blindness, not uncommonly associated with attacks of syncope. Ocular pain is not uncommon after eye-strain, and a slight degree of hypermetropia is very frequent. The fundus oculi may be pallid; the vessels are generally transparent, and give a bright reflex to light. Pulsation in both arteries and veins may be noticeable in the region of the optic disc. Swelling of the disc is sometimes present; hæmorrhages and optic neuritis may take place, and if the latter be associated with vomiting it may give rise to difficulties in diagnosis. Dermatographia is present in some cases, while others present many stigmata indicative of hysteria. Tinnitus aurium and even deafness may occur in severe cases, but disappear with recovery from the chlorosis. Neuralgias are frequent causes of distress, commonly in the area of distribution of the fifth nerve, and particularly severe in the supraorbital branch.

Intercostal neuralgia may be troublesome, and is generally situated on the left side with severe pain, referable to the mamma and præcordia; superficial tenderness in the areas affected is nearly always present. Other neuralgias in the iliac regions and the lower part of the back are met with. One of the principal symptoms of the disease is amenorrhœa. Chlorosis is rare in girls prior to the period at which menstruation usually commences; should menstruation have commenced, the onset of chlorosis invariably disturbs it, either resulting in a scanty pallid flow without regular periodicity, or even complete suppression. Leucorrhœa is common. There are, however, a certain number of cases in which menstruation may occur too frequently, and with excessive loss. It is said that during severe chlorosis with prolonged amenorrhœa the possibility of impregnation is lessened. Polyuria is frequently present in chlorotics, the urine pale and of lower specific gravity than normal; otherwise there are no special clinical alterations.

Chlorosis is generally regarded as an afebrile disease, but careful records of the body temperature in cases over prolonged periods, show that a certain degree of pyrexia is not uncommon, occurring at indefinite periods, and not dependent on any recognizable complication.

The course and intensity of the disease varies very much. It may develop rapidly and with such severity as to constitute an acute condition; generally it is chronic, but very amenable to treatment. It may persist for a long time, with remissions and relapses for many years.

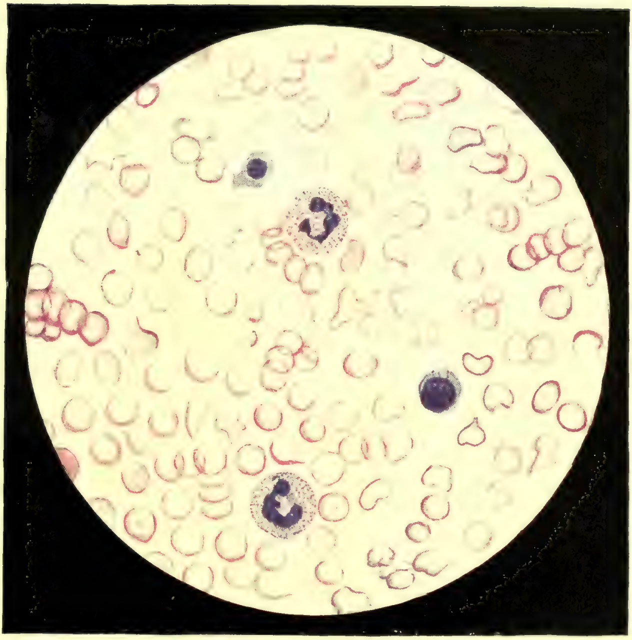
The Blood in Chlorosis

As in secondary anæmia, one may find the drop of blood from the peripheral circulation flow slowly or freely; in chlorosis it generally exudes easily and is pale and watery-looking. The principal change so characteristic of the chlorotic state is the disproportion in the decrease of erythrocytes and hæmoglobin, the latter always falling much below the former. In some cases they fall together and with but slight difference between them, or the erythrocytes may be numerically altered to a trifling degree, while the colouring matter is at a low ebb. In any case the erythrocytic loss is never great. One may find the percentage of erythrocytes at 80 per cent. of the normal, and the hæmoglobin percentage as low as 30. In all cases the colour index is low, sometimes remarkably so. The disproportion between erythrocyte and hæmoglobin loss is present in any stage of the disease, but is generally most marked in a first acute attack, and when nutrition remains good, while it is not so pronounced during recurrences. In cases when the erythrocyte count becomes much reduced, some cause should be looked for to account for it other than pure chlorosis. The low index presents a valuable clinical contrast to the high index of pernicious anæmia. Although this alteration is found clinically in the exuded drop, the total volume of the blood, as revealed by the carbon monoxide method, is increased, and as the oxygen-carrying capacity of the blood remains normal, the increase must be due to excess of plasma and cellular elements, red and white, but each erythrocyte is of low hæmoglobin value. The total volume is increased proportionately to the severity of the disease, and in some cases has reached almost double the normal. The reason of the low hæmoglobin percentage in the exuded drop is due to the lack of colouring matter in each corpuscle and the increase of plasma.

The specific gravity falls with the lowered percentage of the hæmoglobin, and is regarded by some observers as of equal clinical value. Phosphates, potassium and iron are lessened, while calcium and magnesium are increased. The alkalinity is usually normal, or but slightly below it. The isotonicity of the plasma is rather below normal. Coagulation is rapid in chlorosis.

The morphology of the cellular elements need not differ greatly from the normal, with the exception of the pallor of the erythrocytes and their reduced affinity for stains. Their contour may remain unaltered, but their size is generally smaller than normal. In advanced cases all grades of poikilocytosis may be present. Microcytes are uncommon and megalocytes rare. Normoblasts appear at irregular intervals; few in number, they are never a conspicuous feature, and the occurrence of megaloblasts is a curiosity associated with extreme cases. Normoblasts are regarded as significant of regeneration. The staining capacity of the erythrocytes is proportionate to their

PLATE XIII.



RMBuchanan

hæmoglobin content, and is usually feeble; only in severe conditions does polychromatophilia or punctate basophilia occur.

The blood platelets are increased in number. The number of leucocytes remains about normal, and the proportion of whites to reds unaltered. Any leucocytosis which may arise should be regarded as indicative of some complication. The normal count in chlorosis is in direct contrast to the leucopœnia of pernicious anæmia, or the increase found in secondary anæmias associated with suppuration, etc. Should leucopœnia occur in chlorosis, which it does sometimes in severe cases, it is of unfavourable import.

A relative lymphocytosis is usually present in well-marked chlorosis, the neutrophiles being decreased. Eosinophiles may be slightly increased or absolutely and relatively diminished in number. The total leucocytes in the blood is raised, but the fact is not recognizable in the blood drawn from the peripheral circulation, owing to the excess of plasma.

Treatment of Chlorosis

In no other disease associated with a disordered blood state are such brilliant results obtained by treatment as in chlorosis. The return to health is often rapid and permanent. There are a certain number of cases, however, which, from complications, resist treatment or are very slow in their response to it. Happily they are few. In view of the fact that chlorosis is so common a disorder and occurring at a peculiar phase of development, it is sometimes possible to prevent its occurrence by due care for the nutrition and well-being of the child prior to the advent of puberty. This may best be assured by due attention to the diet, which should be regulated towards the gradual deposition of fat by a liberal supply of cream, butter, bread and potatoes; and further by a sufficiency of meat combined with green vegetables rich in iron, and fresh fruits. Abundance of fresh air, with periodic holidays on the mountains or at the seaside, is essential. The body should be cleansed by regular bathing in water not too cold. A girl's education should be so modified, as to allow time during the educational period of life for exercise regulated towards good physical development, and whenever possible such exercise should be taken in the open air. Girls should be treated as children for as long as possible, and the process of transformation into "young ladies" postponed. The use of the corset should be delayed until after the bones of the thoracic cage have become well developed.

Constipation should be guarded against by the judicious use of vegetables in the diet and a regularity of habit. It is doubtful if the administration of iron acts as a prophylactic; it does not seem to possess the same power towards preventing chlorosis as it does in curing it when it is established.

Although the foregoing principles may be exercised with a hope of prevention, there are many instances in which the disease develops in spite of such precautions. On the advent of chlorosis and when the condition is established active treatment is necessary for the purpose of curing it, whether it be slight or severe.

In the majority of chlorotics one of the first principles of treatment is rest, or at least the prevention of fatigue. In well marked cases absolute rest in bed will materially aid and hasten the effect of all other efforts toward curing the condition. In those not so severe, gentle walking exercise short of fatigue may be allowed. Prior to taking a meal after walking exercise, a full hour's rest should be insisted upon. This can best be obtained by the patient retiring to the bedroom and resting on the bed with windows wide open, or reclining on a chair in the open air. This rest hour will allow the circulation to settle down, any exercise temperature to fall, and bring the system generally into a condition best fitted for digestion and assimilation. The general surroundings of the chlorotic should be healthy and bright, ensuring a plentiful supply of fresh air day and night. In cases not requiring absolute rest, it is good for them to go to bed early and get up late. As the health returns more exercise may be allowed, but not sufficient to produce palpitation, dyspnœa or fatigue. Daily lavage of the body and gentle massage is often very beneficial. As the chlorosis subsides, a change of air and locality, with a sojourn for a few weeks in a district of medium altitude, is advisable; but in severe cases more harm than good may be done by residence at very high altitudes, for, although there may be evidence of increased blood formation, e.g. polycythæmia, in health, the cardiac distress and dyspnœa caused at such elevations are undesirable in chlorotics. Chlorotics are very subject to disturbances of the digestive system, varying from the mild type of flatulent dyspepsia to the more intense symptoms associated with gastric ulcer. It is important that any such irregularities should be rectified early, and to this end the diet should be modified in a way conducive to easy digestion and assimilation. In addition to this the administration of stomachic remedies may be necessary, of which there are many combinations, useful according to the peculiarities of individual cases. They generally comprise alkali mixtures with some or other laxative. I find the following generally useful:—

℞.	Sodii Bicarbonatis Sodii Sulphocarbolatis Bismuthi Carbonatis	ā ā ʒij
	Tinct. Rhei co.	℥j
	Pulv. Tragac. co.	ʒj
	Spr. Chloroformi	ʒj
	Aquæ Menthæ Pip.	qs. ad ℥vj
M. ft. m.		

A tablespoonful of this mixture may be taken in an equal quantity of water half an hour before meals, or if there be acidity with "heart-burn" and flatulence, it should be taken one hour after meals. As a rule I find it more useful after than before food. As the digestion improves a more liberal diet may be allowed. The meals should be taken at regular intervals, but on no account should large quantities of slop food be allowed, and as is so often done, given at short intervals. Three meals a day properly chosen will be quite sufficient. Farinaceous foods are inadvisable, being bulky, likely to ferment in the stomach, and tending to aggravate the dilation which is so often present. Further, they are poor in iron. Milk may be taken at meals, but not in such quantities as to interfere with the proper consumption of iron-rich materials. Meat should be taken at least twice a day, underdone, and it is most desirable that it should constitute the largest portion of the patient's breakfast. A good meal in the morning tends to lessen the possibility of syncope later in the forenoon. A most useful adjunct to the dietary is the so-called "black-pudding," which contains a considerable quantity of hæmoglobin in its composition. They can be made without undue admixture of fat. They are inexpensive for patients whose circumstances debar them from purchasing the more elegant organic preparations of iron. Green vegetables and fresh fruit are desirable, because they both contain iron and tend to prevent constipation.

While on the subject of diet, it is hardly necessary to emphasize that all solid food should be eaten slowly and thoroughly masticated, and to this end any defects in the teeth must be rectified. It is essential that the teeth should be kept clean, and any oral sepsis should be rigorously treated, as its presence seriously interferes with recovery, and prolongs the chlorotic condition. There has been much difference of opinion in reference to the desirability of the administration of alcohol in chlorosis. There is little or no evidence of any value in support of it, and much against it. The old idea that "red" wine is good for chlorotics rested largely, I think, on the fact that it and blood laid claim to similarity of colour, but there it ends. Attacks of sudden syncope may be met by the judicious administration of some diffusible stimulant at the time, but the ingestion of a good meat breakfast will lessen the possibility of such attacks.

The medicinal treatment of chlorosis is centred in the administration of iron in some form or other, and this in easily assimilable form and in comparatively large quantities. It is regarded as the specific remedy. Preparations should be chosen which are least likely to derange digestion, the milder forms at first may later on be substituted by the more powerful. Reduced iron, the carbonate or sulphate all have their place. The brilliant results from the administration of Blaud's pill is well enough known. They should be freshly prepared, or the constituents put up in a form which answers the same purpose. In whatever manner the metal be administered, it must be in full doses, after meals, regularly and not intermittently (as is so often the case) for a period of six weeks or more ; the dose should be gradually increased to a maximum by the middle of the course, and as gradually decreased. After two or three weeks' rest it should be resumed. In some cases when the ordinary preparations of iron disagree, recourse may be had to organic preparations such as ferratin, carniferrin, hæmol, hæmatogen, etc.

It is desirable in many cases to administer some laxative during the course of iron, and it may be combined with it or taken two or three times a week in the form of saline aperient water early in the morning.

In spite of such treatment however, there are a limited number of cases which fail to respond, or have a recurrence which is difficult to overcome.

For such, and even in the ordinary cases, arsenic has proved valuable, and may be given in combination with iron. I find the following pill very useful.

℞.	Ferri Sulph. Exsic.	gr. ii.
	Ferri Arseniatis	gr. $\frac{1}{12}$
	Ext. Nucis Vomicæ	gr. $\frac{1}{4}$
	Ext. Belladonnæ	gr. $\frac{1}{4}$
	Ext. Aloes	gr. $\frac{1}{8}$
M.	ft. pil.	

One of these pills may be given after each meal. As one desires to increase the number given to obtain the maximum of iron, the other constituents may be proportionately lessened.

In chlorosis from the outset, and especially in cases which may fail to respond to ordinary treatment, a sojourn at a Spa where chalybeate waters can be obtained may be advised. These are sometimes dilute enough to be tolerated by the empty stomach and easily absorbed. They are best taken fresh at the spring, especially the carbonated waters, which easily decompose. No doubt the change of air and scene which a visit to a Spa entails has its advantages. Among such Spas may be named Schwalbach Spa, St. Moritz (too high), Tunbridge Wells, Cheltenham, Melksham, Trefriw. The waters of the latter springs are extremely rich in iron, need only be taken in small quantities, and are most efficacious in the treatment of chlorosis and chronic secondary anæmias. The village of Trefriw is situated in one of the most beautiful valleys of North Wales; there are two springs, one about double the strength of the other, and it is generally desirable to commence with the more dilute water first. If arsenic be requisite in combination with iron recourse may be had to Levico or Roncegno waters.

In addition to the generally accepted treatment of chlorosis by the administration of iron, attention may be paid to the relief of obesity or tendency to œdema, by the use of a dry diet, limitation of foods tending to produce fat, of fluids, and salt. A salt free diet in such cases may prove beneficial. Sweating may be induced by baths and diaphoretics, and diuresis should be encouraged. Efforts to reduce obesity should be secondary to hæmatinic treatment, but may be useful in conjunction with it. The result will be a more rapid relief of dyspnœa, palpitation and fatigue. The severe headache which some chlorotics experience is best treated by absolute rest. Should severe exacerbations or neuralgias require immediate relief, special remedies, as antipyrin, phenacetin may be given, but intermittently and only when absolutely necessary; calcium lactate is often useful. In very intractable cases lumbar puncture has been suggested for relief of the headache. The removal of small quantities of blood by venesection at stated intervals has been recommended, and found beneficial in special cases. It is supposed to act by direct stimulation of the marrow. It need hardly be stated that it should be regarded as a *dernier resort.* Among organic extracts used in the treatment of chlorosis, good results have been obtained by the administration of bone-marrow or extracts of it. Ovarian and splenic extracts have also been given with success.

PERNICIOUS ANÆMIA

This form of anæmia stands out from all others both clinically and hæmatologically and presents problems in its etiology which in many cases have baffled solution. Brief reference has been made to the disease in a previous section. One particular form of anæmia so resembles the pernicious variety in its symptoms and blood picture, that it may be regarded as an example of the disease, the cause of which we know, and by proper treatment it can be cured. I allude to bothriocephalus anæmia due to the absorption of the products of the decomposition of the parasite, which results not only in hæmolysis but by its action on the bone-marrow in megaloblastic blood formation. The relation of parasite and anæmia has been established experimentally by the inoculation of extracts of the former, so that here we can speak of a variety of pernicious anæmia the etiology of which is known.

There are many conditions which from their association with pernicious anæmia have been regarded as more or less causal factors in its etiology. Whether they are truly causal or simply coincidental or what their relation to the disease is has not been definitely established. The association of pregnancy and parturition with this anæmia is peculiar, a certain number of cases seem to originate in relation to it either during or shortly after gestation, some may be preceded by loss of blood, severe vomiting and diarrhœa, others arise during gestation without any definite cause, and progress fatally.

The anæmia associated with repeated small hæmorrhages may occasionally transit into the progressive grave type; fortunately this is a rare occurrence. Even chronic anæmia of this type may be so severe that the hæmoglobin and blood count falls below that usually noted in the pernicious variety ; but it still retains its normoblastic cytology, and so can be distinguished. There has been a history of syphilis in a certain number of cases. Referring to the atrophic processes of the gastro-intestinal mucous membrane, these may be coincident, not causal, and even the same may be said of cases associated with cancer of the stomach in which atrophy of the mucous membrane is present. Gastric or other carcinoma may be looked upon as definitely related to the anæmia, if by metastes to the bone-marrow the effect is to produce a megaloblastic anæmia. It must not be forgotten that pernicious anæmia may develop during the period of gastric cancer, or the latter may arise during the course of pernicious anæmia. Dr. W. Hunter has advocated very strongly the opinion that one of the causal factors in this anæmia is an infection, of streptococcal nature, of the mouth or alimentary tract. Oral sepsis is not at all uncommon in these cases, and streptococcal ulceration of the tongue sometimes occurs. There is a peculiarity associated with the oral sepsis that I have often noted, that solution of the dentine takes place just above the margin of the gum, so that the teeth become guttered transversely for a depth of about one-sixteenth of an inch. There are cases, however, of pernicious anæmia in which no oral sepsis can be discovered, and many more cases of extreme oral sepsis which do not develop pernicious anæmia. It is difficult to say with certainty whether the oral and intestinal sepsis cause the anæmia, or whether they arise because of it and the lessened resistance to infection which its presence may entail.

Mental shock and worry are also regarded as having some effect towards the production of the disease. It is strange, however, that once the disease is established, patients exhibit a marked indifference to circumstances which are usually causative of anxiety or worry.

It may be that further research may solve the problem of the etiology of this disease, and that it will be eventually placed in the category of secondary anæmia ; until then such cases for which no definite cause can be discovered must be regarded as essential or primary.

Symptoms

No better general account of the symptoms and course of this disease can be given than the one by Addison, who first described it and who called it idiopathic anæmia. "It makes its approach in so slow and insidious a manner that the patient can hardly fix a date to the earliest feeling of that languor which is shortly to become so extreme. The countenance gets pale, the whites of the eyes become pearly, the general frame flabby rather than wasted, the pulse perhaps large, but remarkably soft and compressible and occasionally with a slight jerk, especially under the slightest excitement. There is an increasing indisposition to exertion with an uncomfortable feeling of faintness or breathlessness in attempting it; the heart is readily made to palpitate; the whole surface of the body presents a blanched, smooth and waxy appearance; the lips, gums and tongue seem bloodless, the flabbiness of the solids increases, the appetite fails, extreme languor and faintness supervene, breathlessness and palpitations are produced by the most trifling exertion or emotion; some slight œdema is probably perceived about the ankles; the debility becomes extreme—the patient can no longer rise from bed; the mind occasionally wanders; he falls into a prostrate and half-torpid state, and at length expires; nevertheless, to the very last, and after a sickness of several months' duration, the bulkiness of the general frame and the amount of obesity often present a most striking contrast to the failure and exhaustion observable in every other respect."

The foregoing account covers the general clinical course of the disease. There are, however, certain symptoms associated with the various organs which may be mentioned in further detail. The pallor of the skin may pass into a peculiar yellow or icteric tinge and occasionally pigmentation occurs not only in small patches but over large areas. I have noticed it in several cases, and in one, a female, it was very marked, the sister of the patient presenting similar pigmentation with leucodermia, but no development of pernicious anæmia. I have also seen pigmentation of the buccal mucous membrane. Some œdema of the eyelids or about the ankles is generally present and may remain even during a remission; it is regarded as an unfavourable sign. Small hæmorrhages take place from the mucous membranes due to fatty degeneration of the vessels. Pain and tenderness of the bones, especially the tibiæ and sternum, is not uncommon. The nutrition remains good, there being no loss of weight. The temperature of the body is subject to variations, so that periods of pyrexia occur, but are not proportionate to the severity of the anæmia. Cardiac distress and palpitation are constant symptoms; hæmic murmurs are to be heard over all valve areas, they may be soft or harsh, vary in intensity from day to day, are rarely diastolic, and their intensity is not directly relative to the blood count. The heart may be dilated, and pain and tenderness of the precordia present. As a general rule, however, the cardiovascular symptoms vary in accordance with the anæmic state. Venous murmurs are present in nearly all cases. The pulse tension is low and the frequency changes with slight exertion.

Apart from the dyspnœa there are rarely any special symptoms associated with the respiratory organs, occasionally hæmoptysis may take place, or even hæmorrhage into the pleural spaces.

The appetite is very capricious, there may be complete anorexia and loathing of food. The mucous membrane of the mouth and tongue become very sensitive to acids or condiments; punctate hæmorrhages, vesicles, and desquamations may occur, and I have seen large superficial ulceration of the tongue. Epigastric pain and tenderness, with pulsation are usually present. Vomiting is frequent and may become uncontrollable, hæmatemesis may occur and be profuse. Free hydrochloric acid may be diminished or absent. There may be either constipation or diarrhœa, and the latter is sometimes distressing. The liver may be enlarged and tender, the spleen remains normal unless there has been malaria. The lymphatic glands are not enlarged. The urine may not exhibit any special feature, but often is high-coloured from a high percentage of urobilin. The amount of urea varies. Occasionally there may be slight albumin, or even peptone or albumose. Hæmaturia sometimes occurs. Leucin is almost constantly present and tyrosin occasionally. Uric acid may be increased, and in some, sugar, diacetic acid and acetone is found. Iron and phosphates are both said to be increased. Symptoms referable to the nervous system point to implication of the brain, spinal cord and peripheral nerves. Headache, vertigo and insomnia are not infrequently distressing. Loss of interest in surroundings and indifference to the progress of the disease is manifest ; mania, melancholia, and transient delirium are occasionally met with, and coma, sometimes lasting for several days, often precedes death. Immobility of the pupils, transient hemiphlegias or paralyses, loss of memory and peculiar aphasic attacks may take place. Tabetic and spastic symptoms follow changes in the spinal cord ; rarely there may be peripheral neuritis, but tingling sensations, numbness and paræsthesia are not uncommon. The anæmia may precede or follow the nervous symptoms, and it may improve while they progress ; as a rule once the changes in the nervous system are evident, although slight at first, they are progressive and prove fatal at a comparatively early period. Of symptoms associated with disturbances of the special senses, those referable to the eyes are the most important. They comprise subjective sensations such as flashes of light, but coarse derangements of vision are rare. Subjective symptoms appear before the ophthalmoscope reveals any gross changes in the fundus. Hæmorrhages take place, into the conjunctivæ and retinæ. Pallor of the disc, the fundus generally, and the vessels with tortuosity of the veins can be seen. Circular and triangular hæmorrhages occur in the neighbourhood of the vessels and disc ; they vary much in size, may become partially or wholly absorbed, leaving white opacities. Retinal hæmorrhages are generally more marked towards the fatal termination.

Ear symptoms comprise tinnitus, pulsation sounds, and gradual loss of hearing even to complete deafness. Occasionally there may be loss of smell and taste.

The course of the disease is irregular and the duration variable. Remissions and relapses occur again and again, and the condition may drag on for many months or even years. Some cases progress so rapidly that they may by contrast be termed acute. The intermissions are a remarkable feature. Even when a patient seems at the lowest ebb, exhausted with incessant vomiting and diarrhœa, with a blood count as low as 200,000 per c.mm., and after the onset of coma the symptoms suddenly and unexpectedly cease, and the blood rapidly recovers itself. Palpitation, hæmic murmurs, slight œdema, and the characteristic megalocytic blood picture with polychromasia and punctate basophilia remain, and a relapse occurs sooner or later. Complications are uncommon.

The Blood in Pernicious Anæmia

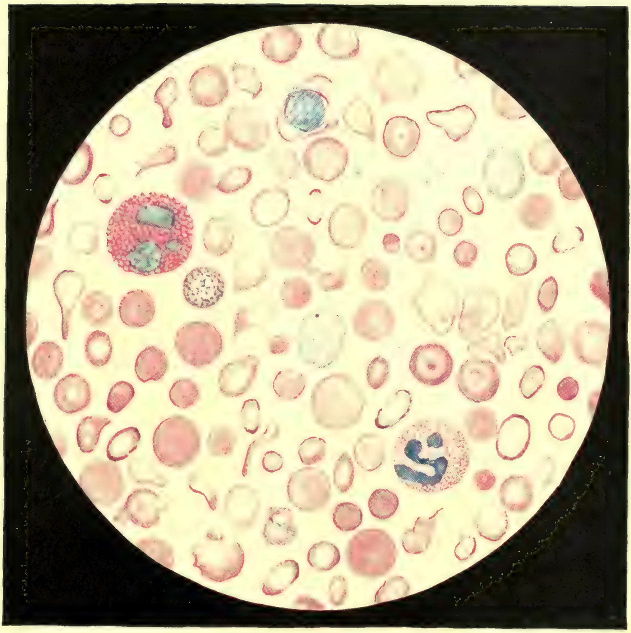
In this disease it is sometimes difficult to obtain an even flow of blood from a finger prick, but it is by no means rare to find the blood exude easily and continue to flow for a longer period than is usual in health. The colour of the drop varies with the gravity of the anæmia, it may appear of good colour or very pale, and with the red cells clumped together and floating about in the plasma. On exercising pressure to obtain sufficient blood for examination, if the flow be feeble, the plasma may pass out more freely than the corpuscles, which would lead to error in the results. The whole volume of blood, as measured by the CO. method, is reduced in some cases, raised in others, the latter being usually associated with an aggravation of symptoms experienced by the individual. By the same means the total hæmoglobin is found to be reduced, so contrasting with chlorosis. The percentage of hæmoglobin in the peripheral circulation is generally low, but with few exceptions does not fall below that of the erythrocytes, so the colour index is higher than normal. This is a very characteristic feature of pernicious anæmia. If, however, repeated examinations be made, the colour index will be found to vary even within short periods. In some it may be high, in others normal, or even below it. During a remission the erythrocyte count increases more rapidly than the hæmoglobin, or even pass beyond that of health. In one case, from a count of 2,000,000 erythrocytes with hæmoglobin at 35 per cent., equivalent to a colour index of 0·87, they rose to 6,000,000, with 80 per cent. of hæmoglobin, colour index 0·6, in the course of five weeks; but in a future and fatal relapse the colour index rose and remained above normal to the end.

It is not uncommon for the colour index to register 1·5. In pernicious anæmia the erythrocytes are numerically decreased, a common average is 1,500,000 per c.mm. in typical cases, but they may fall much lower. The high colour index is accounted for by the individual corpuscles being of greater bulk. In this way, with a low count they may contain sufficient hæmoglobin for physiological purposes compatible with life; and it is surprising what an individual can do even when the erythrocyte count falls as low as 500,000 per c.mm. The total proteids of the serum and the whole blood is reduced, particularly the latter from loss of erythrocytes. In individual corpuscles the proteid content is increased in proportion to their greater size. Water is increased in both corpuscles and serum. Sodium chloride is greater, while potassium and iron are less than normal. The specific gravity and the total alkalinity are both diminished. Coagulation is retarded, in some cases to a minimum, the blood evincing no disposition to separate into clot and serum; this feature is regarded as a differential diagnostic sign between the blood of this and other anæmias. The loss of coagulability accounts in some measure for the occurrence of hæmorrhages in this disease. The erythrocytes are easily affected by hypotonic solutions, and are very susceptible to hæmolysis. The serum itself is hæmolytic to normal erythrocytes.

The morphology of the blood is changed qualitatively. The principal deviation from the normal is manifested by alterations in the size of the erythrocytes. Increase in size is the characteristic change, producing a megalocytic blood picture, many reaching twice the size of a normal red cell. This increase in size of the erythrocytes contrasts with the small forms usually present in other anæmias.

The percentage of large cells varies ; I have seen specimens in which they were nearly all of this type. The percentage of hæmoglobin varies proportionately to the number of megalocytes ; and during a remission, when they become less numerous, the colour index falls. The erythrocytes lose their concavity up to a certain point ; in rare instances they may appear quite spherical. This alteration is better seen in the counting chamber than in stained films. Their colour may not deviate much from normal, but many are richer than usual. The megalocytic blood picture is always evident during active periods of the disease, less so during remissions. Poikilocytes appear in great diversity of size and shape, but generally large ; many are oval, others horse-shoe, pear-shaped or quite irregular. Microcytes are present, but they are not in any way characteristic of this particular disease. Necrobiotic changes are visible in many erythrocytes irrespective of size or shape. Polychromasia is common ; with stains containing methyl blue and eosin such cells may be a light violet or even a distinct blue, with methylene blue and iodine the erythrocytes in this disease exhibit a green colour not usually met with in other forms of anæmia. Basophile granulation or stippling is a marked feature of many erythrocytes, especially during active phases of the disease, less so during remissions. It is seen also in erythroblasts, occasionally when dividing by mitosis. The general affinity for stains is feeble, with the exception of those rich in hæmoglobin. Oval forms exhibit bipolar staining, and cells may exhibit peculiarities of staining as irregular as their shape. Normoblasts and megaloblasts occur ; the former are never numerous, they may appear alone or accompanied by the latter. Megaloblasts may be few or many, more often few, and prolonged search has to be made for them, but their presence is an important feature. They may be present in one film, absent or scarce in another made from the same drop of blood. They generally increase numerically before death, but I have sometimes failed to find them during the last stages of the disease, but the blood at the same time presenting a megalocytic picture. The importance of the presence of

PLATE XIV.



Buchanan

erythroblasts rests with their kind rather than number; normoblasts are not uncommon in any severe anæmia, and are indicative of a regenerative process, but megaloblasts are very characteristic of pernicious anæmia, and their presence, apart from their number, which fluctuates day by day, is both diagnostic and prognostic, pointing to a degenerative change associated with the bone marrow. Careful search, therefore, should be made for them. It is not necessary to count them, as I have said they fluctuate, and no accurate count can be made from stained films, because this can only be done indirectly, admitting many possibilities of error, and providing only very approximate results. In order to count them, they must be compared numerically with the leucocytes present. The number of the latter per c.mm. is obtained by the Thoma-Zeiss apparatus in the usual way, and the number of erythroblasts counted in a stained film, at the same time counting all the leucocytes seen in the fields examined. The calculation is then made by means of the following formula—

$$\left.\begin{array}{l}\text{Leucocytes}\\\text{per c.mm.}\\\text{(Th. Zeiss)}\end{array}\right\} \times \frac{\text{Erythroblasts counted in film.}}{\text{Leucocytes counted in film.}} = \left\{\begin{array}{l}\text{Number of}\\\text{erythroblasts}\\\text{per c.mm.}\end{array}\right.$$

If the ratio of leucocytes to erythrocytes be known the ratio of erythroblasts to erythrocytes is evident. If desirable, the number of megaloblasts and normoblasts may be recorded separately, the proportion of one to the other is important.

Between the normoblast and megaloblast may be found intermediate or atypical nucleated cells, attention to which has been drawn in a previous section.

Although the erythrocytes tend to form clumps in a thickly spread film, characteristic rouleaux are absent, especially in advanced cases; due, I think, to the loss of concavity of the individual corpuscles.

There is an absolute decrease in the number of leucocytes, leucopœnia being the rule. Any increase above normal numbers must be regarded as due to some complication. The count may average 2,000. Lymphocytes are increased relatively, and there may be an absolute increase towards the close of the disease. A striking feature is the absolute and relative decrease of polymorphonuclear neutrophiles. Sometimes the coarse eosinophile cells may be relatively increased. I have noted this in severe cases. Occasionally myelocytes may appear. Blood platelets are few in number and fibrin formation is lessened. The morphological changes which give the blood picture the stamp of pernicious anæmia during an active phase may disappear almost completely during a remission, so that it might be difficult to form a definite opinion if the blood be examined then for the first time. During the remission, while the blood is returning to the normal, the colour index falls, the abnormalities of the erythrocytes become less and less conspicuous, normoblasts become more numerous than megaloblasts; the erythrocyte count rises; the leucocytes increase numerically by influx of polymorphonuclear neutrophiles, myelocytes disappear, and lymphocytes become fewer. However much the change may favour the normal picture, it is rare for megalocytes to disappear entirely. By prolonged search a megaloblast may be discovered, and this with megalocytes, though numerically few, is a most important diagnostic feature, for the principal character of the blood in this disease is that it is both megaloblastic and megalocytic.

Treatment

It should be pointed out, before discussing the treatment of pernicious anæmia, that the peculiar intermittent character of the disease indicates a tendency towards natural recovery, although the remissions be but transient, and that apart from any form of medication, natural efforts, though abortive, are made to overcome the cause of the disease, whatever it may be.

Thus there is hope that as we learn more about the true cause, our efforts to overcome the condition by therapeutic measures will eventually prove successful. During recent years the outlook has been more promising, and a few instances of recovery have been recorded, while the remissions, even in cases which have eventually proved fatal, have been materially prolonged and relapse delayed.

Were we cognisant of the cause in every case, the possibilities of successful treatment would be enhanced; and as an example of this may be mentioned the effectual result obtained in cases due to bothriocphalus latus, in which the removal of the worm by medication and purgation is followed by recovery.

The principal remedy for the disease is arsenic, which was first introduced by Byrom Bramwell in 1877. Under its influence improvement rapidly takes place, especially during the first course of treatment by it. A few cases are said to have been permanently cured by it.

Relapses are delayed, and even when they take place improvement may follow again and again, from the judicious administration of arsenic, and so a relapse be suppressed. It is usually given in the form of Fowler's solution, commencing with a small dose of two minims gradually increased until even fifteen or twenty minims are taken three times a day by the end of three or four weeks. The maximum dose may be kept up for a few weeks and then gradually lessened. Occasional small doses should he administered throughout the remission. It should always be given with food, and signs of intolerance should be noted such as gastric irritation, vomiting and diarrhœa. Their advent demands a reduction of the dose or complete cessation of the drug for a few days.

One need not adhere rigidly to Fowler's solution, *Liquor Sodii Arsenatis* may be used. If more convenient the *Acidum Arseniosum* may be given in pill. The drug may be given hypodermically as *Liquor Sodii Arsenatis* or *Arsenate of Iron* (Martindale) but there does not seem to be any advantage to be gained by it. Some physicians do not push the Fowler's solution to such large doses as twenty minims, but prefer smaller doses administered regularly without intermission during the whole course of the disease, without discontinuing it during apparent recovery. In this way relapses are said to have been delayed for years.

Cacodylic acid and cacodylate of soda have also been used as substitutes for the usual preparations of arsenic, but it is doubtful if they possess any special advantages.

If thought desirable the arsenical waters such as Roncegno, Levico, or Guberguelle may be used. The administration of iron has no effect upon the anæmia, and is supposed in some cases to do harm. It is stored up in the internal organs where siderosis is already present.

Phosphorus and quinine have fallen into disuse, and oxygen inhalations are next to useless. Arsenic remains the most important remedy. Although its action is that of a hæmolytic and causes anæmia, at the same time its stimulating action upon the bone-marrow far exceeds its hæmolytic power, and so fresh cells are produced rapidly and at the same time they are more resistant. The use of bone-marrow introduced by Sir Thomas Fraser is beneficial from time to time. It may be given fresh, in the form of sandwiches, on in the form of tabloids or glycerine extracts. It should be given in large quantities, several ounces daily. It should be persevered with, but patients dislike it after a time. A useful and elegant way of giving marrow is in jelly. The marrow should be made into a paste with port wine, three of marrow to one of wine, about a drachm of gelatin is dissolved by the aid of heat in sufficient water, mixed with some glycerine, and the marrow paste added.

This preparation is easily taken, and tolerated for long periods. A simple way is to mix the marrow and wine paste with one of the flavoured jellies ordinarily used at table. The action of marrow may be a dual one. In the author's opinion its effect is produced by direct marrow stimulation, and by bactericidal or antitoxic action. Transfusion of defibrinated human blood, or by the direct method, have been tried many times in the treatment of this disease. The results have been remarkably favourable in some cases, ineffectual in others. How it acts is difficult to explain; the amount of blood used has been small, not more than two or three ounces, and the effect may be due to the action of the plasma as an antitoxic agent rather than to the corpuscles. The administration of fresh horse serum may prove useful.

Transfusion should be reserved until other remedies have been tried without effect, or in severe cases with threatened collapse, or coma. In view of the theory promulgated by W. Hunter, who regards the disease as an infective process due to streptococcus, an antiseptic method of treatment has been suggested and practised with promise of good result. It is directed towards the removal of oral, gastric and intestinal sepsis, supplemented by the use of antistreptococcic serum. All deficient and decayed teeth should be removed, and some form of antiseptic mouthwash, such as dilute solution of formol with peroxide of hydrogen rigorously applied; the mouth should be cleansed frequently.

Gastric antisepsis should be persevered in, by the use of salicylic acid which is recommended by Hunter as the most efficacious and generally useful. Perchloride of mercury, salicylate of bismuth, charcoal or carbolic acid have all been found useful. Stomach lavage may be found beneficial. Intestinal antisepsis may be aimed at by the use of perchloride of mercury, small doses of calomel, benzo-naphthol, urotropin, etc., or the bowel may be irrigated with weak potassium permanganate solution. In addition to these measures the injection of 5 or 10 c.c. of polyvalent antistreptococcic serum, given at intervals of a few days, is said to be very beneficial, especially in cases while the disease is in an early stage. Should the serum injections be objected to, it may be given by the mouth early in the morning on an empty stomach, or by rectal enema. Early diagnosis and early treatment is important. In addition to antiseptic measures arsenic should be administered. The general treatment consists of attention to the diet, so that the most nourishing food may be chosen, which is easily tolerated and digested, without causing gastric irritation. The dislike to meat often renders it necessary to fall back upon farinaceous and vegetable diet, but it is not advisable to give too much " slop " food. Rest is absolutely essential during the severe stages of the disease, mental work or worry should be avoided, and vicissitudes of external temperature should be guarded against, as people suffering from the disease are very sensitive to excessive heat or cold.

		NORMAL BLOOD.	SECONDARY
			POST HÆMORRHAGIC.
Hæmoglobin		100 per cent.	Decreased, falls with erythrocyte loss, but does not return to normal at the same rate.
Erythrocytes.	Number per c.mm.	5,000,000.	Moderate or great loss, proportionate to hæmorrhage.
	Colour	Characteristic oxyphile.	Normal at first, pallor of cells later.
	Size	Regular.	Average diameter lessened.
	Pockilocytes	None.	Present in severe cases.
	Polychromasia	None.	Present, early.
	Basophilia	None.	Uncommon.
	Megalocytes	None.	Variable, from absorption of fluid.
	Microcytes	None.	Common.
	Normoblasts	None.	Few or abundant. Blood crisis may occur.
	Megaloblasts	None.	Rare. Occasionally in severe cases.
	Colour index	1.	First normal, then low.
Leucocytes.	Number per c.mm.	8,500.	First relative, then absolute leucocytosis.
	Polymorphonuclear	70–90 per cent.	Increased.
	Lymphocytes	20–30 per cent.	Transient increase in some cases.
	Eosinophiles	0·5–4 per cent.	Variable.
	Myelocytes	None.	Rare, only in severe cases.
Blood platelets		—	Increased.
Coagulation		—	Increased.

ANÆMIA.	PRIMARY ANÆMIA.	
Chronic.	Chlorosis.	Pernicious Anæmia.
Variable. May be proportionate to erythrocyte loss or fall much below it.	Marked decrease, disproportionate to erythrocyte loss. Average 50 per cent., may be lower.	Marked decrease, average 25 per cent. Proportionately high to erythrocyte loss.
Moderate loss most common, may be excessive.	Never great loss.	Always markedly decreased, often down to 1,000,000.
Pallor of cells, staining affinity irregular.	Pallor of cells marked and proportionate to Hb. content. Staining affinity poor and irregular.	Pallor variable, many cells high coloured. Staining affinity varies.
Average diameter lessened.	Average diameter lessened.	Average diameter increased.
Present, variable, marked in severe cases.	Present in advanced cases.	Very marked, large.
Present in severe cases.	Occurs only in severe cases.	Very common.
Present in severe cases.	Occurs only in severe cases.	Very common.
Absent.	Absent. Very rare even in severe cases.	Characteristic feature.
Present in severe cases.	Not common.	Not so common.
Present, fluctuate, not reliable indication of the severity of the anæmia.	May occur in severe cases, never a conspicuous feature.	Present in variable numbers.
Rare, occasionally present.	Very rare even in severe cases.	Characteristic feature, number variable, not necessarily numerous, predominating erythroblast.
May be normal, more commonly less.	Always low; average 0·5.	Usually high, 1·3 to 1·7.
Leucocytosis present.	Normal, ratio of W. to R. unaltered. Increase a sign of complication. Leucopœnia may occur in severe cases, an unfavourable sign.	Decreased, 4,000 or less. Increase a sign of complication.
Increased.	Decreased.	Decreased.
Relatively decreased, may be increased in severe cases.	Relative increase.	Relative or absolute increase.
Relatively diminished, may be increased.	Absolutely and relatively decreased.	Absent. Sometimes a relative increase.
Occasionally found in severe cases only.	Absent.	Occasionally present. In severe cases may be constant.
Generally increased (inconstant).	Increased.	Decreased.
Increased.	Increased.	Decreased.

The foregoing table gives a brief diagnostic survey of the anæmias which have individual characters differentiating them one from another. It is not usually difficult to locate them correctly, but there are instances in which the blood may require repeated examination in order to arrive at a correct conclusion. The principal feature of chlorosis is the low colour index, due to marked loss of hæmoglobin, unassociated with any marked alteration in the leucocytic count. In chronic secondary anæmias the blood may simulate the chlorotic type, but in contrast to it there is some increase of leucocytes. Pernicious anæmia presents a blood picture peculiar to itself, the principal features being the high colour index, the megalocytic and megaloblastic type of erythrocyte associated with leucopœnia. In some cases of cachexia such as are met with in malignant disease and syphilis, especially the former, the blood may approach so closely that of pernicious anæmia as to be almost indistinguishable from it, but in these the colour index is low rather than high, megalocytes if present are few rather than many, and normoblasts are more numerous than megaloblasts, if the latter be found at all; and further, leucocytosis, not leucopœnia, is the rule. There are, however, other anæmias which resemble so closely pernicious anæmia that they may be rightly classified as such, I refer to bothriocephalus anæmia and such as are due to hæmolytic poisons. Nitro-benzole and arseniuretted hydrogen are good examples. Providing the blood change has not proceeded too far, the removal of the cause in these cases will be followed by a return of the blood to normal; this is particularly noticeable in bothriocephalus anæmia, without of necessity the tendency to relapse so common in true pernicious anæmia. Eosinophilia, which is so often present in the anæmias associated with helminthiasis, is a useful diagnostic feature. Although from the blood picture such varieties demand classification with pernicious anæmia, they must be regarded as instances of this, the cause of which is known.

“APLASTIC” ANÆMIA

Under this heading are included those rare conditions associated with profound erythrocyte and hæmoglobin deficiency in which the blood picture departs from the ordinary types, and reveals an absence of any of their usual characteristic features. The microscopical examination of the blood indicates a lack of function on the part of the bone-marrow, as if it were at its lowest ebb, which it really is, for the autopsy reveals an aplasia of this tissue which corresponds with the blood picture. Erythroblasts are absent, and leucocytes, especially the polymorphonuclear neutrophiles are markedly diminished, so that several microscopic fields may have to be searched in order to find a single leucocyte. This condition is associated with the appearance of purpuric eruptions and hæmorrhages from the mucous membranes, resembling purpura hæmorrhagica. A similar blood state may arise in cases of pernicious anæmia towards the end of the disease. I have seen it in this disease shortly before a fatal termination, but the erythrocytes retained their megalocytic character.

SPLENIC ANÆMIA

This disease, which years back had been regarded as a form of pseudoleukæmia, has now been allotted a place as a clinical entity. The principal feature of it is primary splenomegaly which is chronic and progressive. Associated with this is anæmia of secondary or chlorotic type, and of varying grade and intensity, in some cases entering upon the domain of the pernicious. The blood shows no leukæmic change, and leucopœnia is the rule, although a leucocytosis may occur, but symptomatic of some intercurrent complication. No cause for the disease is known, and it is not correlated with such diseases as malaria, syphilis, alcohol, cirrhosis of the liver, leukæmia, or such like. It is supposed to be due to an intoxication of unknown nature. There is marked family tendency and heredity. It is essentially a chronic condition lasting for many years; in one of my cases the splenomegaly was discovered thirteen years ago, and the patient, a male, still enjoys fairly good health, and pursues his occupation daily. There is a great tendency to hæmorrhage from the stomach or bowels, and I have noticed coincident with it an immediate collapse of the spleen. In one case it shrunk from the iliac crest to be just palpable below the ribs. Enlargement follows again, and in a few days it will have returned to its usual large size.

The body temperature fluctuates considerably, and may become hectic in character, especially in the late stages of the disease. Melanoderma is not uncommon. As the disease progresses there comes a terminal stage with cirrhosis of the liver, jaundice and ascites, at which period it has been designated Banti's disease. The lymphatic glands are not enlarged. The blood changes may be summed up as anæmia, with normal leukocyte count, or commonly leucopœnia.

The *erythrocytes* vary in numbers. In some cases there may be a fairly high count, but generally there is profound loss, the average being about 3,000,000, but it may reach as low as 1,000,000, rarely below this. Abnormalities in the form of poikilocytosis, endoglobular degeneration, and polychromatophilia appear, and in grave cases with anæmia of pernicious type, megalocytes may be numerous. Nucleated red cells, either normoblasts, intermediate forms or megaloblasts are present; the former are the more common, and outnumber the latter, which are present in the severe anæmia. The number of erythroblasts varies; they may be few or abundant, and even " blood crises " may take place.

The *hœmoglobin* percentage is low, and out of proportion to the erythrocyte loss, so that the rule is a colour index below unity, although in exceptional cases it may be above it occasionally.

The *leucocyte* count, may be normal but is usually low. Often below 5,000 they may fall to half that or even less. There is no constancy in the differential count, but a relative increase of lymphocytes is common, with a corresponding relative decrease in polymorphonuclear neutrophiles. Unless the anæmia be profound, myelocytes very rarely appear.

The eosinophiles remain unaltered, and occasionally an increase of basophile cells is observed.

Any increase of polymorphonuclear neutrophile leucocytes is generally due to intercurrent complication.

Treatment

Medicinal treatment is not satisfactory. Iron does not seem to be of much value, arsenic has not the same effect as in other anæmias and the leukæmias. The administration of perchloride of iron and calcium chloride have been found useful, along with intestinal antisepsis. Hepatic extract and red bone-marrow have been used with benefit.

The general health must be sustained. Hæmorrhages should be treated on lines previously mentioned. Splenectomy has proved successful in several cases, and if it has not been directly curative it has prolonged life. When there is ascites other operative measures may be taken with a view to establishing collateral circulation.

Exposure of the spleen to the X-rays may be useful in reducing its size when it is causing pain.

ERYTHRÆMIA

The term erythræmia is used to denote a disease characterized by a persistent increase in the total number of erythrocytes in the blood, an increased total volume, in fact a true plethora, as compared with a relative increase of erythrocytes or erythrocytosis. The term is analogous to leukæmia as representing a persistent total increase of leucocytes in comparison with the transient condition of leucocytosis.

It is also known as Vaquez's disease, Osler's disease, myelopathic polycythæmia, polycythæmia rubra vera, and erythrocytosis megalosplenica.

A glance over these terms reveals three main elements in the disease which have given rise to them, namely, an increase in the number of erythrocytes, enlargement of the spleen and bone marrow change.

It is a peculiar disease, characterised clinically by redness and cyanosis of the face and extremities, headache, mental torpor, vomiting, some albuminuria, a tendency to hæmorrhage from the mucous membranes, especially of the stomach and intestines, increased blood pressure, a progressive asthenia, and enlargement of the spleen.

The disease seems to be more common in males than females, and occurs about the middle period of life; the condition is essentially chronic and may last for years. The blood changes are manifested by a marked increase in the number of erythrocytes even to over 11,000,000 per c.mm.

They exhibit much variation in size, microcytes and macrocytes being present. The fact that in normal blood the erythrocyte volume comprises about 50 per cent. of the total, it would seem almost impossible to find room for twice the normal number per c.mm. without the blood becoming practically solid, but this is permitted by the extreme variation in size and the number of diminutive forms.

There is no doubt that the blood is less fluid; it flows sluggishly, spreads under the cover slip slowly, and exhibits an increased viscosity and specific gravity. The total volume is greater than normal, even exceeding twice the usual amount, so that there is a true plethora. Changes in staining reaction are common, and there may be a fair number of normoblasts. The colour of the blood is darker than usual, probably due to deficient oxygenation and corresponding to the cyanosis.

The hæmoglobin is proportionate to the erythrocyte increase and may reach 200 per cent.

The leucocytes may be present in normal proportion, or there may be a leucopœnia relative to the number of erythrocytes. There is usually a high percentage of polymorphonuclear neutrophiles.

Few opportunities have occurred for investigation of the post-mortem appearances and morbid anatomy. The blood-vessels throughout the body have been found universally engorged; thrombotic infarction of the brain and spleen, fibrosis of the heart, and hypertrophy of the spleen are the usual findings, and in some cases deposits of tubercle have been present in the latter. The bone-marrow presents evidence of increased activity, towards the generation of erythrocytes, but no particular change has been observed in leucocyte formation. The hyperplasia of red marrow extends into the shafts of the bones. Whether the erythroblastic activity is analogous to tumour formation, or due to some toxic influence which stimulates the marrow to increased erythrocyte formation, with or without a coincident mild hæmolysis remains to be determined.

Treatment

The general health must be attended to; exercise must be taken in a moderate way. Headache may be treated by nitrites which have been found useful for this purpose. Arsenic has done good in some cases, but it has aggravated the condition in others. Large doses of quinine have proved beneficial. X-ray treatment has no effect. Inunction of biniodide of mercury ointment over the spleen with exposure to artificial heat has been tried with benefit. Venesection of comparatively large amounts of blood at intervals seems to hold out the greatest prospects of good. Citric acid or citrates may be administered with a view to reducing the viscosity of the blood.

CHAPTER X

LEUKÆMIA—MYELOID LEUKÆMIA—MIXED-CELLED LEUKÆMIA—LYMPHOID LEUKÆMIA—CHLOROMA—LYMPHADENOMA.

LEUKÆMIA

LEUKÆMIA is a disease characterized by a persistent increase of leucocytes in the blood, associated with gross clinical changes in the spleen or lymphatic glands or both, and with hyperplastic changes in the bone-marrow. Originally two distinct forms were recognized clinically, one in which enlargement of the spleen was a special feature, the other in which enlargement of the lymphatic glands was the principal concomitant. This classification was based on clinical grounds rather than on the blood changes, until Neumann showed that the bone-marrow was the principal hæmogenetic organ, when this classification became impossible for in both forms the marrow has been found changed. The association of the blood picture and marrow changes with splenic enlargement gave rise to the term spleno-medullary for this leukæmia, while the term lymphatic was retained for the variety associated with glandular enlargement. Both forms of leukæmia, however, may show clinical features in common; the so-called spleno-medullary may be associated with glandular enlargement, and the lymphatic form with hypertrophy of the spleen, so that the anatomic symptom-complex is not a constant in either case. The opinion is gradually gaining ground, and I think rightly, that both are referable to primary changes in the bone-marrow, so that they are histogenetically in close relation, while clinically they may differ to a certain degree. The classification must rest upon the blood picture, which by the more recent and approved methods of examination forms a more accurate basis than clinical examination alone.

In one the blood picture with myeloid characteristics and the marrow changes correspond, so that this variety has been designated myelogenous or myeloid leukæmia. In the other the blood presents a so-called "lymphocytic" or "lymphoid" picture and the marrow does the same.

Intermediate between these are those peculiar mixed varieties, which present clinically and hæmatologically features common to both. Not only this, but as I have personally noted, a typical myeloid leukæmia may change through the mixed variety to a characteristic lymphoid type, with aggravation of all symptoms, resembling acute leukæmia with early fatality. In this case the spleen, glands and marrow were all affected, the first two at early and later periods respectively, and the marrow consisting almost entirely of lymphoid cells. In view of such cases one must regard the marrow as the primary seat of origin, the other organs being secondarily affected. Where we have, on the one hand, a marked lymphoid blood picture, with little or no affection of lymphatic glands, it seems strange that in cases which come under the category of pseudo-leukæmia, e.g. Hodgkin's disease, in which there is such pronounced glandular affection there should be little or nothing characteristic of a lymphoid blood picture.

In discussing the origin of leucocytes (chap. vii.) I drew attention to the view, with which I am in accord, that the mononuclear cell prevalent in the blood of acute leukæmia is not the same as the hæmal lymphocyte, but in my opinion is an early stage in the life history of the myelocyte, a "leucoblast." The change then in the bone-marrow, in acute lymphoid leukæmia, would represent a proliferation so marked, that the cells are thrown into the blood stream in an immature form, before their development into any typical variety as seen in the adult cells has been accomplished. On the other hand, a profound leucolysis may be present sufficient to destroy cells before maturity.

In one case which changed from the myeloid to the lymphoid type, treatment by X-rays had caused the temporary disappearance of the ordinary myeloid blood picture to be followed at a later date by the acute lymphoid type over which the same treatment had no control. The action of the X-rays produced a marked leucolysis, as revealed by marked degenerative changes in the leucocytes. They became more vulnerable to the technique of examination, films were difficult to obtain without much damage to the cells in spite of the greatest care in manipulation. Many cells had become achromatophilic, and there was a striking deficiency in reaction to basic stains, so that even the nuclei of polymorphonuclear cells stained badly.

What the ultimate nature of the marrow changes in leukæmia blood states may be is practically unknown. Its relation to cell proliferation, as evidenced in malignant neoplasms, has been much discussed, in reference to which I have been struck by the extraordinary resemblance, of films made from round-celled sarcoma by puncture, spread and stained, to the cells present in lymphoid leukæmia.

It may be that future and more exhaustive investigations may reveal connective links between the well recognized leukæmic blood states and those which by their uncertainty and inconstancy of hæmatological change are classified as pseudo-leukæmias.

The rare diseases known as leukanæmia and chloroma present peculiarities which offer a wide field for investigation. Their morphology will be described under their appropriate headings.

Leukæmia may occur at any age from infancy to senility, but is most common at the middle period of life. Males seem to be more often affected than females. It is a chronic disease, but acute cases are met with occasionally. The more acute it is the more likely it is to be of the lymphoid type. The particular cause of leukæmia is unknown in spite of the most approved methods and searching investigation. The enthusiasm of the bacteriologist has at times almost persuaded us that a micro-organism is the primary factor in its origin. When we consider those cases which run an acute course, resembling in many ways specific infective fevers;

the fact that Obrastzow recorded an attendant upon a leukæmic patient as having developed the disease and died of it ; that the disease is so often preceded and accompanied by lesions of the alimentary tract from above downwards, permitting the entrance of some infective virus, indicate the possibility of its origin being founded upon an infective process. Several observers have associated different organisms with the disease, but none of those found have fulfilled the laws necessary to establish their claim to its origin. Löwit[1] described a parasitic body in the blood, which he termed the *hœmamœba leukœmiœ*, but its presence has not been substantiated by other observers, but regarded as an artefact due to technique. A certain number of cases give a history of previous malaria, others that the onset followed injury. Heredity may have some predisposing influence. In women the disease has been found to develop during pregnancy or at the climateric.

In one case a leukæmic mother passed through five pregnancies and bore non-leukæmic children ; her grandmother, mother, and one brother suffered from symptoms suggestive of the disease. Four of her children subsequently died of leukæmia. On the other hand, a leukæmic child may be born of a healthy mother. The lower animals may suffer from the disease, it has been met with in dogs, horses, oxen, swine, cats and mice. In reference to a classification of the varieties of leukæmia on a hæmatological basis, it is to be noted that clinically their progression may be acute, subacute, or chronic, and with these divisions the blood picture to a certain extent corresponds. Thus the myeloid form is generally the most chronic, the lymphoid the most acute, and the mixed-celled intermediate between them. Symptomatically they have much in common.

MYELOID LEUKÆMIA

Symptoms

This form is usually chronic in duration, but occasionally it may be acute.

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[1] *Die Leukämie als Protozoeninfektion,* Wiesbaden, 1900.

Its onset is, as a rule, insidious, so that it is difficult to determine the period at which it may have commenced. The first symptoms of which the patient complains, and for which medical advice is sought, are usually dyspnœa, palpitation, pain or discomfort in the left hypochondrium, anorexia, dyspepsia, distention of the abdomen, enlargement of one or other group of lymphatic glands, or pains in the bones and sternum. For a considerable period there may be no disturbance of nutrition or noticeable anæmia, and the patient remains in ignorance of the serious state of affairs. Anæmia shows itself sooner or later, and becomes more severe as the disease progresses. The skin and mucous membranes assume a peculiar pallor, the skin having a dusky, sallow or even slightly bronzed appearance. There is a marked tendency to hæmorrhages from the mucous membranes, and repeated and often profuse epistaxis is sometimes the initial warning of the underlying condition.

Hæmorrhage may take place from the gums, stomach, bowels, into the skin retina, internal organs, serous membranes or brain. On examination of the abdomen the spleen will be found enlarged, not only by an increased area of splenic dulness but by palpation. It varies in size, and may extend below the level of the umbilicus; I have seen it reach the pubes; the surface remains smooth and the notch is easily palpable; its consistence is firm. There may be pain and tenderness over it, and occasionally friction can be felt and heard; on auscultation also a splenic souffle is sometimes present, and the organ has even been felt pulsating. The size of the spleen is variable at different times in the same case, and may rapidly reduce in size after a severe hæmorrhage or diarrhœa. The liver becomes enlarged in some instances more than others; it remains smooth; ascites and peritonitis may both be present. Œdema of the feet or legs is not uncommon. Enlargement of lymphatic glands when it occurs is more often associated with the axillary and inguinal groups than the cervical. They are rarely tender, show no tendency to break down or infiltrate the skin. Should internal glands enlarge, they may produce pressure symptoms according to their situation, upon bronchi vessels, etc. Sometimes the lingual glands and tonsils may enlarge. The disease generally runs a subfebrile course; there may be periods of apyrexia alternating with pyrexial attacks of a remittent type. In some, intermittent fever with profuse sweats may occur. The urine exhibits no special peculiarities other than excess of urea and uric acid; hæmaturia is a rare complication.

Symptoms associated with the cardiovascular system are few, hæmic murmurs are present with the anæmia, the heart's apex may be raised by the splenic enlargement. Thrombosis of the vessels in not common, but it does happen occasionally, and may even give rise to gangrene. Hæmopericardium may occur.

Further than the dyspnœa there are no special respiratory symptoms, there may be bronchitis or pneumonia, and I have seen tuberculosis of the lungs in one case. Hæmoptysis may occur apart from tuberculosis and may be profuse. Occasionally one meets with pleuritic effusion and hæmothorax. Gastro-intestinal symptoms are more or less always present; nausea, thirst, vomiting and attacks of diarrhœa may be distressing.

Hæmatemesis may be alarming or fatal, but hæmorrhage from the bowel is not frequent. There may be ulceration of the bowel simulating dysentery.

Apart from the pallor of the skin, it is often dry, lustreless, and the seat of erythema, urticaria, acne, furunculosis or purpura; in the later stages of the disease profuse perspirations may occur.

Symptoms referable to the nervous system are generally due to local hæmorrhages. Headache is frequent. There may be facial paralysis, deafness, vertigo and tinnitus simulating Meniére's disease. Alterations in vision may arise, and are similar to those present in profound anæmias. Retinal hæmorrhages have been mentioned, but the ophthalmoscope reveals in addition a leukæmic retinitis with white and cream-coloured elevated spots on the retina due to leucocytic deposits.

The most serious complication associated with the nervous system is cerebral hæmorrhage, which not infrequently is the immediate cause of death.

Loss of virility, amenorrhœa or menorrhagia in women, and persistent painful priapism due to coagulation in the corpora cavernosa, are symptoms associated with the sexual apparatus.

The course of the disease is not constantly progressive; there are periods of variable duration when it seems to remain at a standstill, but sooner or later relapses occur, and with increased anæmia, the harassing abdominal discomfort, or from complications such as severe hæmorrhage, hydrothorax, ascites, repeated attacks of diarrhœa, distressing headache, the store of vitality becomes exhausted. Attacks of syncope, gradually progressive asthenia and emaciation, mark the progress of the disease, and the end may be ushered in by attacks of delirium, mania and coma, or the occurrence of cerebral hæmorrhage, after a duration of a few months or years from the period at which the disease was first recognized.

The Blood in Myeloid Leukæmia

The exuding drop of blood as obtained for microscopical examination, may not differ much in colour from normal, or it may appear rather opaque or even creamy, but the latter is an extreme change rarely seen.

It flows easily, but when spread in a film takes on a granular or greasy appearance, particularly so when there is marked leukæmia, due to the great excess of leucocytes.

In the fresh specimen, the leucocytic increase is self-evident, and on examination for some time, more so if on a warm stage, it will be noted that comparatively few cells exhibit active amœboid movement. Many cells appear larger than are seen in a normal blood film and more granular, but apart from these peculiarities it is not easy to differentiate the different varieties unless a dark ground illumination be used. It is necessary to stain the film by one of the approved methods previously described, in order that the characters of the leucocytes be displayed.

The numerical increase of leucocytes may be very great;

in one of my cases they amounted to 1,300,000 per c.mm., with a ratio of 1 white to 2 reds.

The number fluctuates at different times, and great differences may occur in the course of a few hours. The actual number, however, may not denote any increase, so that one might be led astray by the count alone, unless stained specimens be examined.

By the use of stained films the qualitative alteration in the blood picture is rendered visible, and it is this that is of so great importance in the diagnosis of the disease.

In addition to cells common to normal blood, adventitious cells appear which are foreign to it and only occur on comparatively rare occasions in other diseases. The following varieties of cells appear which go to make up the characteristic blood picture of myeloid leukæmia.

Myelocytes.—The characters of these cells have been described previously. Their presence puts the stamp upon the disease. Their numbers may represent the greatest percentage of all leucocytes present ; 60 per cent. is not uncommon, but they may fall as low as 5 per cent. As long as they are present, even in small numbers, they indicate the persistence of the disease. They are usually the largest form of leucocyte present, but they vary very much, and all sizes are met with, down to the dimensions of an ordinary lymphocyte. Transitional stages are seen showing alterations in nuclear contour ; some cells are less granular than others, and some are found in transit to neutrophile or eosinophile cells, as the case may be. I have noted many instances of myelocytes containing mixed granulation, oxyphile and basophile occurring side by side.[1] This may be a phase in the life history of the cell.

Myeloplaxes.—I have observed these in rare instances in sections of myeloid leukæmic blood.

Eosinophile cells.—These are absolutely increased, even to great excess over normal, but relatively to the total leucocyte count the percentage may be greater or less than usual. They are such a striking feature in the blood picture that at one time they were regarded as distinctive of leukæmia, but in view of the fact that marked eosinophilia occurs in other diseases, they are no longer accounted as pathognomonic of this disease. The large majority are mononuclear, but all sizes and shapes appear, from quite large to dwarf forms. Variations in the shape of the nuclei are common, and transitional varieties between the large mononuclear and the polymorphonuclear form normal to the blood can be distinguished. Cells in transition from myelocytes to eosinophile cells, and with varied degrees of granulation occur.

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[1] *Journ. of Pathology and Bacteriology,* Dec., 1896.

Basophile cells.—These cells are, with few exceptions, a constant feature of blood in myeloid leukæmia. They appear in all sizes and depth of granulation, so that they resemble on the one hand the ordinary basophile cell of the blood with fine basophile granulation, or on the other are of larger size with fewer but larger granules, some of which stain pure with the basic dye, others metachromatically. Occasionally these cells are larger than any others present. These cells are not rendered visible by Erlich's triacid stain, but are very evident in films stained with methylene blue or dahlia. Their number varies, is absolutely increased, and they represent from 1 to 10 per cent. of the leucocytes. In some cases they may outnumber the eosinophiles.

Polymorphonuclear neutrophiles.—The absolute number of these cells is increased, but the percentage relative to the others is low. They not only vary much in number, but also in size and other characteristics. Some are large, others quite small, and but for their irregular nucleus might easily be classified incorrectly. They by no means keep to a standard of granulation, so that many are but slightly granular, others devoid of granulation and only recognized by their irregular nuclei. Many transitional stages can be discerned between the myelocyte on the one hand and the "polymorph" neutrophile on the other, not only in amount and depth of granulation, but in the gradual alteration in the shape of the nucleus—the so-called "transitional neutrophiles."

Lymphocytes.—These cells, though they may be absolutely increased, are relatively much diminished, and take a subordinate position in the blood picture of myeloid leukæmia. The small variety may be reduced to under 1 per cent. The so-called large lymphocytes are present in greater numbers than the small variety. I think, however, that many of these larger cells will have to be regarded as of myelogenous origin, and distinct from hæmal lymphocytes. Atypical lymphocytes have been described which are difficult to classify, in that they present certain characters by which they resemble myelocytes. When in large numbers these cells, which in my opinion are leucoblasts, lymphoid, or pre-myelocyte cells, give the blood picture a distinct character, so that the leukæmia may no longer be regarded as purely myeloid, but a mixed variety, intermediate between the myeloid and lymphoid.

In many cases mitotic division can be seen; I have only recognized it in the myelocytes proper. In sections of leukæmic blood fixed immediately it is shed mitoses are found very frequently, more so than in spread films.

Erythrocytes.—In the early stages there is not as a rule any marked degree of anæmia, and erythrocyte loss is not a special feature, but later on the count may fall to 2,000,000 per c.mm. Erythroblasts are common, and have no relation to the amount of erythrocyte loss. They may appear in quite large numbers, even with a count of 4,000,000 reds. Of all blood states in which erythroblasts appear, myeloid leukæmia is the most common. Both varieties occur, megaloblasts and normoblasts, the latter usually the more numerous.

Atypical erythroblasts on the borderland between the two are often present, and may give rise to difficulty of classification. Great diversity of nuclei are to be seen, and mitosis is not uncommon. There is no special alteration in shape or size of the erythrocytes, other than would be associated with the degree of anæmia. Polychromatophilia and basophilic stippling both occur, the former especially evident in the erythroblasts.

The ratio of leucocytes to erythrocytes may rise to 1 to 10, or even 1 to 1; but it fluctuates considerably, depending almost entirely on the number of leucocytes rather than deviations in the erythrocyte count.

The estimation of the percentage of hæmoglobin is rendered difficult according to the amount of leukæmia, the white cells interfering with the colour comparisons. In all cases it is diminished and the colour index low.

The coagulation time varies. I have noted coagulation be very slow in blood collected in glass tubes, and there is always a difficulty of separating clot from serum, even in the centrifuge.

The alkalinity is usually decreased and the specific gravity said to be variable; it being difficult, because of the number of leucocytes, to obtain reliable estimations. The number of blood platelets varies. Charcot-Leyden crystals appear in dried films, but have no diagnostic importance.

MIXED LEUKÆMIA

Mixed leukæmia presents a blood picture which is intermediate in character between the myeloid and lymphoid varieties. The distinguishing feature is the presence of many "lymphoid" or leucoblastic cells. It is doubtful if myeloid leukæmia is ever pure and quite free from such cells, or that the acute lymphoid leukæmia is ever quite free from neutrophile myelocytes. In the one the neutrophile myelocyte predominates; in the other the lymphoid cells; the mixed variety being a transitional stage between the two. A typical myeloid leukæmia may pass into the lymphoid, with a corresponding change in the blood picture, and it may be assumed that at a certain stage of this transit, the blood state may be rightly classified as "mixed." These lymphoid cells have been described in a previous section, and need no further comment here. They may vary from a size rather larger than a hæmal lymphocyte to a neutrophile myelocyte. The nucleus, often spherical and large relatively to the cell body, stains feebly with basic dyes; that is, it is more or less achromatophilic, while the cytoplasm constitutes a narrow rim of basophile material surrounding the nucleus, in many instances ex-

PLATE XV.

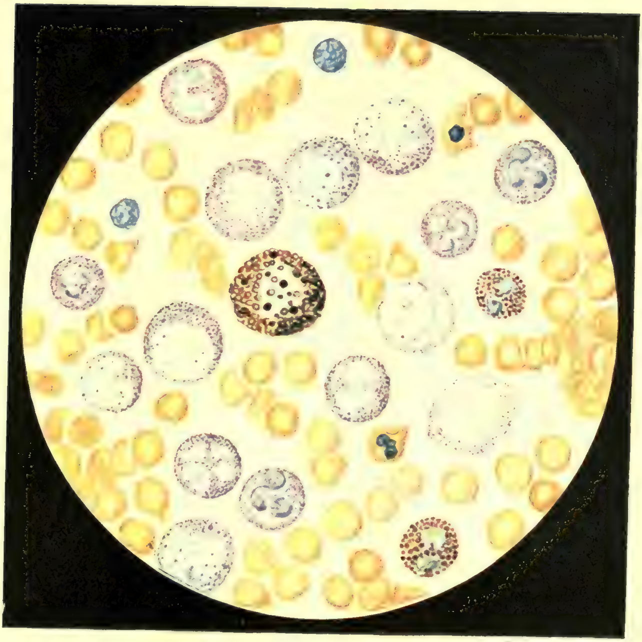

FIG. 1.

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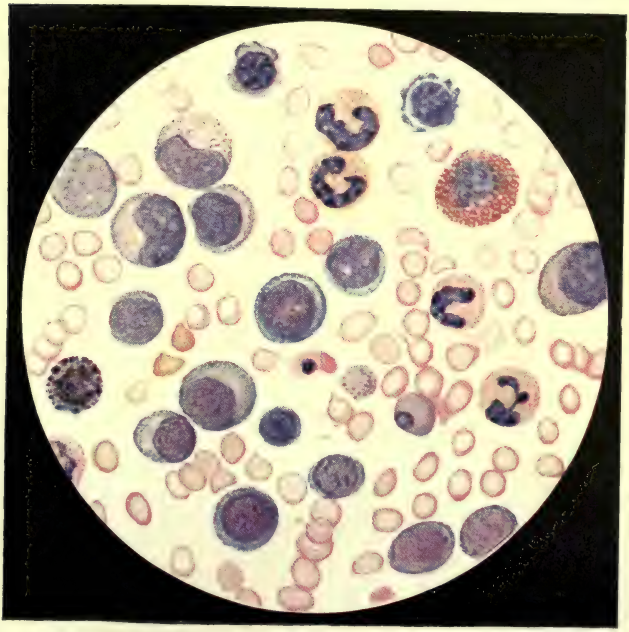

FIG. 2.

RJMBuchanan

truded from the surface in small masses. This variety of cell I described as far back as 1896 as an atypical basophile myelocyte.[1] The large forms are common in lymphoid leukæmia, and constitute the special feature in the disease, and have been usually classified as large lymphocytes.

Treatment of Myeloid Leukæmia

As in pernicious anæmia, so in leukæmia, natural remissions of the disease are not at all uncommon, so that one must keep this fact in mind when estimating the efficacy of any special line of treatment. Endeavours must be made on general principles to sustain the bodily health by judicious exercise or rest, fresh air and liberal diet. In reference to exercise the tendency to hæmorrhage must not be forgotten, and any danger of trauma should be avoided. Apart from symptomatic treatment, arsenic is the drug which exercises a definite amelioration of the condition; under its influence the leucocyte count falls, and the anæmia tends to disappear. Its administration should be carried out on the same lines as for pernicious anæmia. Antiseptic treatment of the alimentary tract from above downwards is advisable.

Quinine has been found useful in cases associated with malaria. Iron may benefit the anæmia, and should be given in conjunction with arsenic. Bone-marrow may be useful for the anæmia. The application of the X-rays has given most surprising results. Amelioration of the condition has been rapid, and great hopes were entertained that in them we had a remedy to hand which would surpass all others. This is so in some respects, for by no other means can such a rapid change in the condition be obtained. The spleen rapidly diminishes in size, and the leucocyte count falls, so that it may even reach subnormal. With this the general condition is improved. It is said that the earlier the case the more marked and rapid the result. Old standing cases do not respond so readily, and repeated courses of treatment for relapses lose their efficacy. In one case which came under my notice the patient was relieved for about two years, but after the return of the disease it was not influenced in the same way by the rays, and the blood picture had changed to the "lymphoid" type; and it has been noted that the rays may do no good in lymphoid leukæmia, but rather tend to aggravate the disease. The X-rays seem to destroy the leucocytes in the body, they become broken up and degenerated; it is difficult for good films to be made, and they respond very poorly to any stains; nuclei become less and less chromatic. With the fall in leucocytes there is at first a corresponding increase in the excretion of uric acid, but this after a time ceases, although the diminution of leucocytes goes on, so that the rays not only break up the cells but inhibit their development and by experiments upon animals it has been shown that they may be used too much, so that the power of the marrow to produce leucocytes may be brought to an end. The rays are also supposed to act by producing a toxin which destroys the leucocytes. It seems that the more fully developed cells are destroyed first; the lymphoid cells are not affected, so that their development into cells of the several series is stopped, and they may not recover this function. Hence the change which may take place from the myeloid to the lymphoid type by the influence of the rays.

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[1] *Journ. Pathol. and Bacteriology,* Dec., 1896.

The rays may be applied over the spleen, the epiphyses of bones, the shafts of the long bones and the sternum. Ten to twenty minutes' exposure at a time over one place or several places in all, is the average duration required, applied every day, every other day, or at longer intervals.

Dr. O. T. Williams[1] has shown that the products formed by the disintegration of the leucocytes by the X-rays may give rise to severe constitutional disturbance with fever, so that the intervals between exposures should be longer and the duration of each exposure shorter than has usually been the case. The excretion of uric acid should be estimated daily as a guide to the length of the intervals. The

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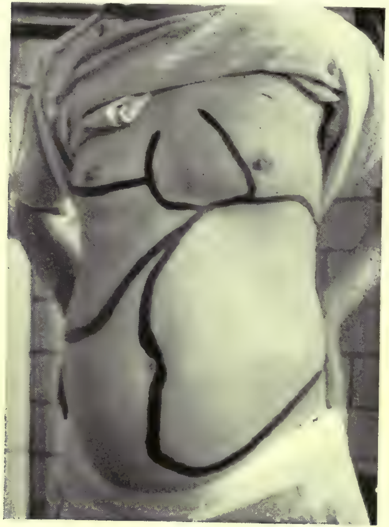
[1] *Lancet*, May 5, 1906.

PLATE XVI.



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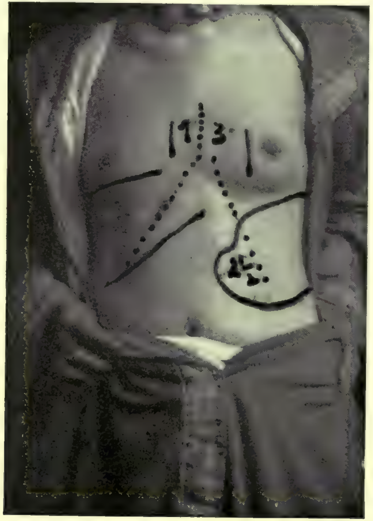

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use of the rays should also be controlled by the blood examination, so that their effect should fall short of producing the lymphoid blood picture. Care should be exercised to prevent dermatitis or burns. The former may pass off after a time, but there is a tendency to the formation of new blood vessels, which produce an appearance resembling capillary nævi. X-ray burns are very obstinate, and take weeks to heal, and the nævoid appearance left behind is very marked, the vessels assuming a stellate arrangement.

Cases have been recorded in which the leukæmic condition was materially altered by the advent of some intercurrent specific febrile infection such as pulmonary tuberculosis, influenza, sepsis, and erysipelas. The last seems to have most effect ; it produces a retrogression of all symptoms and a change in the blood, so that the leucocytes common to the disease are greatly reduced, and the blood picture assumes the characters of a polymorphonuclear neutrophile leucocytosis. In view of these effects, the treatment by vaccine therapy may prove useful, by inoculation with measured quantities of dead organisms, for example, the streptococcus erysipelatosus. I do not know if such has been tried ; it is quite well worth consideration.

Splenectomy is dangerous because of the tendency to severe hæmorrhage, and in any case is not advisable, for, as it has no effect upon the disease, it answers no useful purpose.

LYMPHOID LEUKÆMIA

This disease may occur in two clinical varieties, the **acute** and **chronic.** The points of difference between them are in degree and duration rather than in kind ; they are both characterized by a persistent increase of the lymphoid cells in the blood, with enlargement of the lymphatic glands and spleen to a varying degree, and tendency to hæmorrhages into or from the mucous membranes, and under the skin. There is always a certain amount of anæmia, generally more marked than in myeloid leukæmia.

The etiology of the disease is but little known; it may occur at any period of life, but is more frequent in young people, and seems to be more acute the younger the person. In the acute variety the rapidity of its progress, febrile condition, great prostration, and early fatal termination even before the characteristic glandular enlargement has become conspicuous, although there be a characteristic blood picture, point to the probability of its being an acute infective process. The chronic form, lasting, as it may, for months or even years, with much glandular involvement, points also to an infection, the fatal termination preceded by cachexia being so often due to an exacerbation of the original process similar to the acute.

Attempts have been made to correlate the disease with microbic invasion, but none of the organisms found have been proved to be the cause of it. The "parasitic" body of Löwit has already been mentioned, but its connexion with this variety of leukæmia is discredited, as it has been in the myeloid variety.

There are differences in the clinical course and symptoms of acute and chronic lymphoid leukæmia which call for a separate description.

ACUTE LYMPHOID LEUKÆMIA

Symptoms

The onset of the disease may be insidious or sudden. The individual may feel quite well prior to the time when the disease manifests itself acutely, or there may have been some prodromal symptoms lasting for a few days, comprising weakness, pains in the head and neck, dyspnœa, lassitude, vertigo, fever, pain over the spleen or swelling of the joints, pallor and puffiness of the face with epistaxis, inflammation of the mucous membranes of the mouth, nasopharynx or tonsils.

The principal symptoms which are associated with acute lymphoid leukæmia are the progressive, sometimes rapid, enlargement of the lymphatic glands; the comparative slight enlargement of the spleen, compared with the excessive splenomegaly of myeloid leukæmia; the occurrence of rigors or "chills;" the irregular pyrexia and pains in the bones; the occurrence of ulcerative stomatitis and necrotic patches on the buccal mucous membranes, the tonsils, palate and pharynx; a tendency to hæmorrhages from the mucous membranes generally, and the presence of purpuric eruptions and other blood extravasations of varying intensity in the skin and subcutaneous tissues; and the retinal hæmorrhages which are present in nearly all cases. Associated with these is the pathognomonic blood picture.

The lymphatic glands are enlarged in nearly all cases, but the change is never so great as in the chronic form. They may not be larger than a hazel nut, and may escape notice unless a careful examination be made. The cervical groups are most commonly affected, but the axillary and inguinal glands do not escape. As a rule, they are movable and do not infiltrate the surrounding tissues. They vary in consistence; in some instances are fairly hard, but in others soft and "boggy" on palpation. They are not usually painful or tender, but when there are marked buccal inflammations, may be very tender and even matted. They are not necessarily symmetrically enlarged. Towards the close diminution in size may take place. Cases occur in which the superficial glands show no enlargement. The spleen may not be enlarged, only firm, but sometimes it may become enormous, especially in children. The increase may be measured daily in very acute cases. It is generally of firm consistence and freely palpable over all its borders. Rupture of the spleen has been known to take place. The changes in the mucous membranes of the mouth and nasopharynx may be due to hæmorrhages beneath it, producing swellings; necrotic patches producing sloughing ulcers may be present on the gums, soft palate and pharynx. The teeth may be loosened, and in some instances the condition may simulate noma. The breath becomes offensive. Inflammation and necrotic ulceration of the tonsils is not uncommon, and the side most affected corresponds with the largest mass of cervical glands. Breaking down of the mucous membranes may lead to profuse hæmorrhage. The skin assumes a peculiar pallor and the face becomes puffy, petechiæ from the size of a pinhead, or larger, and even extensive hæmorrhagic extravasations may take place, simulating scorbutus; necrotic areas may follow at the seat of hæmorrhage. The skin is very susceptible to the slightest injury, and a prick, as by a hypodermic needle, may cause a large subcutaneous extravasation. Very slight finger-pressure may produce ecchymosis. The purpuric eruption generally appears first upon the buttocks and thighs. The tendency to hæmorrhage is universally distributed throughout the body, so that not only the skin but any internal organ or cavity is subject to it. Thus, if occurring in the lungs, it gives rise to hæmoptysis and signs of pulmonary apoplexy; in the brain, to hemiplegia, coma and death; in individual nerve nuclei or sheaths to corresponding paralyses, accounting for deafness, vertigo, facial paralysis and bulbar palsy, etc.; in the retina to alterations of vision, the ophthalmoscope revealing local changes similar to those previously described. Hæmorrhage may take place from the intestinal or genito-urinary tract; or into the pleura, pericardium, peritoneum, pancreas or suprarenal capsules. The abdominal symptoms associated with the disease may simulate other acute conditions and give rise to great difficulties in diagnosis. The liver is generally enlarged and tender. There may be diuresis or anuria, albumin present in the urine, and occasionally it may have a green tinge of colour as in chloroma. The excretion of uric acid is increased. Priapism sometimes occurs. The heart's action is increased in frequency, the pulse corresponds and is small. The dyspnœa present is due to affection of the naso-pharynx, pressure from mediastinal glands, concurrent bronchitis or direct implication of the lungs. Pyrexia is a constant symptom; it is very irregular, may simulate typhoid fever or sepsis, and in some cases gradually reaches a maximum towards the end, or may fall rapidly just before death. The course of the disease is generally rapid It may last from a few days to a few months.

Emaciation, anæmia and asthenia are progressive, and death takes place from gradual asthenia, from some complicating hæmorrhage, or coma.

Intercurrent septic infections often change the character of the symptoms; the spleen and lymphatic glands diminish in size and the leukæmia may give place to an ordinary leucocytosis. The prognosis is invariably unfavourable. Remissions have occurred in a few cases, but the termination is always fatal. The disease may simulate so many other affections by symptoms referable to various organs that much difficulty may arise in diagnosis, especially in those acute cases in which the lymphatic glands and spleen are slightly if at all affected. An examination of the blood is absolutely necessary to establish the diagnosis. The following case is illustrative of this. It was of an exceptionally acute form, with a characteristic blood picture, and a symptom-complex which presented features of special interest in relation to differential diagnosis. For the notes of the early condition I am indebted to Dr. Schofield, of Southport, who kindly placed them at my disposal. The history obtained was to the effect that the patient (F., æt. 52), who had previously been quite well, had contracted a "chill" on August 10, 1906, during a menstrual period, and for a few days suffered from an illness simulating influenza, with febrile symptoms, gastric irritation, and relaxation of the throat. This passed off during the week. Nothing further developed until August 17, when the patient again became ill, and the throat symptoms recurred, with some enlargement of the submaxillary and cervical lymphatic glands, pyrexia, and vomiting. On August 24 severe abdominal symptoms set in in addition. Dr. Schofield, who saw the patient for the first time on this date, relates that she was well nourished; she was confined to bed, restless, the pulse 84, regular and soft, the temperature 100·2¼°F., the expression anxious, complexion dusky and skin dry. Pain was complained of in the right hip joint and left lumbar region. Headache was troublesome, nausea and vomiting distressing, the ejecta containing streaks of bright red blood. On examination of the abdomen the recti were extremely rigid, and there was pain and tenderness in the epigastrium. There was resonance to percussion all over except the sigmoid region, where it was impaired. In this region there was resistance on palpation, due to accumulated fæces. The use of enemata removed this, with much relief to the patient. There was no splenic enlargement or tenderness. The cervical glands were enlarged on both sides and very tender. The thyroid was also enlarged. The lungs were not affected, and the heart, with the exception of feeble sounds, revealed no valvular lesion. A small quantity of urine was obtained with difficulty, and contained a small amount of albumin. The patient was quite conscious, but occasionally rambled in her speech. Towards evening the pulse, now weaker, rose to 100, and the temperature a degree to 101·2 °F. The abdominal pain became so severe that morphia and atropine were administered hypodermically.

On the morning of August 25 the pulse was weaker and more rapid, though regular, and the pyrexia had reached 102·2 °F. There was great restlessness, and twitching of the hands and shoulders. The cervical glands were more swollen and tender. The abdominal pain had increased; purpuric spots were noticed on the legs and sides of the abdomen. The hypodermic injection had produced two hours' sleep, followed by restlessness, great anxiety and apprehension, which increased during the day.

I saw the patient with Dr. Schofield at 4 p.m. on this day. The first thing noticed was the peculiar dusky complexion and the facies, which was indicative of the intense pain, which caused her to cry out almost incessantly. As the urgent symptoms were indicative of some intra-abdominal lesion, attention was given to its examination first. There was general abdominal distension, with a localized and greater distension in the epigastrium and upper umbilical region. Pain in these regions was intense and constant, but, with severe paroxysmal exacerbations, it was increased by change of posture, and passed through to a corresponding area of the back. The local tenderness here was exquisite, so that palpation was rendered difficult. Over this area the recti were "on guard," but below it the abdomen was not so rigid, but tender in several places. The abdomen was tympanitic all over, and no free gas was present in the peritoneal cavity, so that the liver dulness was not diminished even with the patient lying on the left side. There were no signs of fluid in the abdomen.

Respiratory movement was absent from the abdomen, rendering the breathing shallow and painful. On catheterization, the bladder was found to be empty, and there was suppression of urine. The edge of the liver in the lateral region could be felt one finger's-breadth below the costal margin. Vomiting had occurred at intervals, with increase of abdominal pain during the act. There was no intestinal obstruction, the bowels having moved subsequent to the action of the enemata, and flatus had been passed at intervals. There was no visible peristalsis of the large or small gut, and no "ladder pattern" distension indicative of obstruction of the small intestine.

Rectal and vaginal examinations revealed nothing abnormal. Tenderness was present in the right hip joint, but movements were normal. During the abdominal examination the purpuric eruption was noticed; it was present on the sides of the trunk, the legs and arms; the extravasations varied in diameter from $\frac{1}{16}$ to $\frac{1}{2}$ an inch or more. Where the hypodermic needle had been used, suggilations had formed, and extravasations had occurred from finger pressure during lifting.

The upper cervical and submaxillary glands were enlarged and discrete, but very tender. The gums were seen to be spongy and bled easily; necrotic ulcers were present on the soft palate and uvula; the tonsils were much enlarged, and of a deep purple colour. The back of the pharynx was of a similar colour and swollen. There was little or no pain on swallowing. The thyroid gland was larger than normal and there was some substernal dulness. Another slightly enlarged gland was present in the left axilla and two in the left groin. A faint systolic murmur could be heard at the heart's apex. Physical signs of engorgement were present at the base of the left lung. Owing to the great restlessness of the patient, no examination of the fundi oculorum was attempted.

In view of the enlarged lymphatic glands and the purpura, a blood film was made at the time and examined without staining. An enormous leucocytosis was recognized. This was sufficient to determine the nature of the disease. Specimens stained later on the same day corroborated the diagnosis, and established without doubt the lymphoid type of the leukæmia. That night and the following day the condition of the patient became rapidly worse. I saw her again at 6 p.m. on August 26. The abdominal distension, epigastric and umbilical pain and tenderness were much more severe; the pulse more frequent, hard and small. Respiration or movement of any kind aggravated the pain, especially spinal movement. The purpura had increased, the facies become more strained, the duskiness deeper, and the face was bathed in perspiration. The patient passed into a state of collapse and died at 7.45 p.m. A blood count made one hour and three-quarters before death gave the number of R.B.C.'s at 2,856,000 per c.mm., and a leucocytosis of 528,000—a proportion of 1 white to 5·4 reds. Stained specimens revealed the lymphoid cells to be almost wholly of the large variety.

Differential leucocyte count—

Lymphoid cells . . .	98·6	per cent.
Polymorphonuclear . .	1·2	,,
Eosinophiles . . .	0·2	,,
Lymphoid cells—		
Large	95·6	per cent.
Small	4·4	,,

The symptom-complex present in this case pointed in two different directions: on the one hand, to some severe abdominal lesion; on the other, by the enlarged glands and purpura, to an abnormal blood state.

Taken separately, the abdominal symptoms suggested one of two things: either a perforated gastric ulcer, or an acute affection of the pancreas, probably hæmorrhagic. The previous history did not fit in satisfactorily with stomach perforation, nor did the physical examination exactly denote it. From the agonizing and paroxysmal character of the pain, its increase on change of posture, the extreme sense of anxiety and dread, and the absence of free gas in the peritoneal cavity, opinion inclined towards the acute lesion of the pancreas.

Again, the enlarged glands and purpura, the peculiar necrotic areas in the mouth, the stomatitis and affection of the tonsils, and the bone pain (over the hip), all pointed to an acute infective process associated with an abnormal blood state, compatible with the clinical picture of acute lymphoid leukæmia ; and on these grounds a blood examination was made at once, and the true nature of the condition established.

The next consideration was whether the two conditions were separate and independent entities, or in any way related. To my mind, there was no difficulty in deciding this relation—that the abdominal symptoms were due to a hæmorrhage into the pancreas, the immediate outcome of a hæmorrhage tendency, associated with acute lymphoid leukæmia. An interesting feature was the combination of purpura with critical abdominal symptoms, a condition recognized in the disease known as Henoch's purpura, in which peritonitis, and even perforation of the stomach, have been recorded.

In the case related, however, there was no hæmorrhage from the bowel, such as is usually present in the abdominal crises of Henoch's purpura.

The engorgement of the left lung was interesting, as cases of acute lymphoid leukæmia occasionally simulate and have been diagnosed as acute pneumonia, before the blood examination has revealed the true condition.

Another notable feature was the sudden onset, with rapid progress and early death, after an acute illness of nine days' duration.

Correlative to the acuteness of the disease was the prevalence of large lymphoid cells in the blood.

The severe abdominal symptoms raised the question of exploratory operation. Such a procedure was judged as hopeless, and the blood state rendered it unjustifiable. It is worthy of remembrance, should a similar abdominal condition be met with in future, that the lymphatic glands be examined; and if found enlarged, a blood film be made and examined at once; for an operation under such conditions as have been related above would undoubtedly end in disaster.

CHRONIC LYMPHOID LEUKÆMIA

Symptoms

The onset of chronic lymphoid leukæmia is so insidious that it is almost impossible to record in any given case the time at which the condition first commenced. The lymphatic glands may enlarge and retrogress for some considerable time without any marked degree of ill health, and it is only when an examination of the blood reveals the characteristic changes that the true nature of the condition can be with certainty established. The glandular enlargement may be confined to one group only, or several may be affected at the same time. The cervical axillary and inguinal glands are more commonly noticed, but even with no palpable enlargement of these, post mortem examination will most probably reveal the change in the thoracic, abdominal or pelvic groups. The glands remain separate, soft, elastic, with a consistence similar to that felt on palpation of the testicle. They retain the oval shape, contract no adhesions, do not infiltrate surrounding structures and are rarely painful or tender. The spleen is almost constantly enlarged and sometimes to a marked degree; its increase in size is usually proportionate to the duration of the disease. Its consistence is firm, and it is palpable over all its borders. Changes in the size of both glands and spleen may take place, so that there may be periods of diminution alternating with increase.

Intercurrent infections usually cause a temporary retrogression, and a corresponding change in the leucocyte count and the blood picture. The liver also becomes enlarged, but icterus is uncommon. The skin is often dry, and there may be intolerable itching, with urticarial eruptions, the wheals of which may leave behind small hard nodules, which gradually disappear. Lymphomatous nodules may appear on any part of the body, but they have a predilection for the face, causing disfigurement. The condition known as *lymphodermia perniciosa* begins with itching, redness, and swelling, afterwards followed by tumour formation. The true relation between this condition and the leukæmia is uncertain. Hæmorrhages into the skin and subcutaneous tissues may take place from time to time, but are usually associated with acute exacerbations of the disease. As a rule, however, hæmorrhages are not a common feature in the chronic form of lymphoid leukæmia. Symptoms associated with the nervous system are generally due to growths in the neighbourhood of individual nerves causing paralysis. Ear symptoms may arise common to leukæmia. Ocular troubles consist of lymphoid deposits in the eyelids and conjunctivæ; hæmorrhages may occur into the latter, the iris, ciliary body and retinæ. Leukæmic retinitis is frequent. Dyspnœa and pressure symptoms may arise from infiltration of the nose, pharynx, trachea or bronchi, or from enlarged mediastinal glands.

The course of the disease is marked by progressive pallor and emaciation with pyrexial attacks at intervals. Death is due to interference with the functions of internal organs from pressure, from a fatal pneumonia, gradually increasing cachexia and asthenia, or the onset of an acute exacerbation resembling the variety previously described. Although the course is progressive, remissions take place, and the disease may drag on for a few years. The prognosis is invariably unfavourable and after definite cachexia has become established, it rarely exceeds a year before the fatal termination.

The Blood in Lymphoid Leukæmia

The blood picture in the acute and chronic forms differs somewhat, the difference being rather in degree than kind of blood change.

The blood as it flows from the finger prick appears watery, more so than in myeloid leukæmia, but it rarely has any tendency to creamy consistence, and spreads easily in films. There is generally a more marked anæmia than in myeloid leukæmia. The main feature is the absolute increase of mononuclear cells, which have been designated lymphocytes. These occur in two forms, large and small, and it is generally accepted that the large cells are associated with the acute disease, the small with the chronic, but it is rare to find either quite pure, so that mixed or intermediate cases are not uncommon. There is some reason to believe that acute and chronic lymphoid leukæmia will sooner or later be separated from one another when their histogenesis is more fully known. That the cells which predominate in the acute variety, at any rate, are true lymphocytes is very doubtful. I do not consider them in this category, but regard them of myelogenous origin and as leucoblastic or primitive lymphoid cells of the bone-marrow ancestral to other leucocytes. The following are the hæmatological characters of lymphoid leukæmia.

A leukæmic condition of the blood is associated with erythrocyte and hæmoglobin loss. The ratio of whites to reds varies. The leucocytosis is rarely so great as in myeloid leukæmia. The number of leucocytes may vary from 30,000 per c.mm. upwards. In one of my cases the reds numbered 2,700,000, the whites 900,000, or 1 white to 3 red; in another case reds were 2,856,000, and whites 528,000, or a proportion of 1 white to 5·4 red. Of the leucocytes present the mononuclears far outnumber any others.

Mononuclears (lymphocytes), or lymphoid cells.—These are absolutely and relatively increased. They may be large, small or intermediate in size, and altogether may average 90 per cent. of the total leucocytes. In the second case quoted above they were at 98·6 per cent., and of these 95·6 per cent. were large, 4·4 per cent. small. It is difficult to classify them as small and large in some instances, for many intermediate cells are sometimes present. As a rule, the small variety of lymphocyte identical with the hæmal

PLATE XVII.

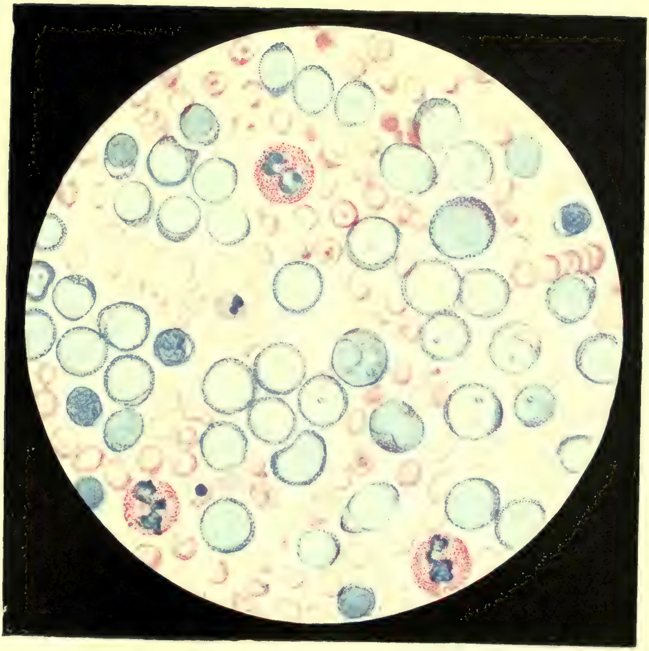

FIG. 1.

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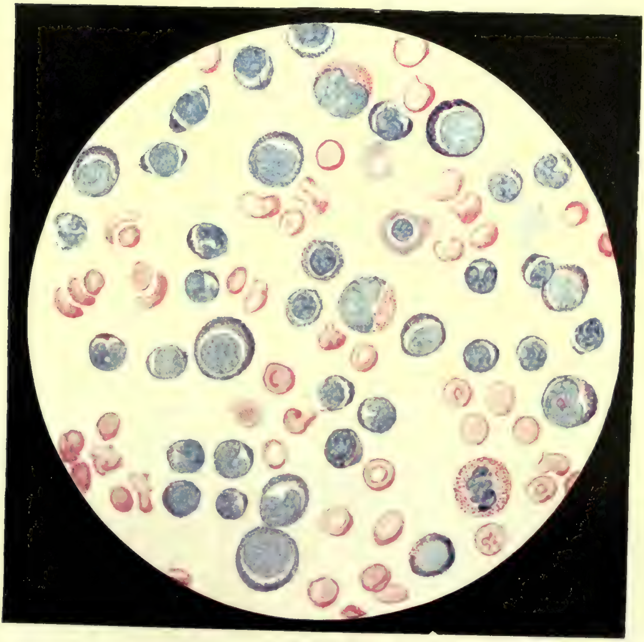


FIG. 2.

RMuirBuchanan

cell is not increased, and certainly relatively diminished. It is doubtful if the large and small cells are of identical origin.

Polymorphonuclear neutrophiles.—These are absolutely and relatively diminished, and, where there is a great excess of lymphoid cells, in a microscope field polymorphonuclears are conspicuous by their scarcity. They are of the ordinary type and present no special peculiarities.

Eosinophiles are rare, their percentage relative to the others is very low, but occasionally the total may be greater than normal.

Neutrophile myelocytes.—These are generally present, but few in number, representing a small percentage of all the leucocytes.

Basophile cells.—These do not constitute a special element in the blood picture of lymphoid leukæmia, although they may be increased. I have occasionally seen them well in evidence in this disease.

Erythrocytes.—There is always a definite fall in the number of erythrocytes and, in acute cases, may average 2,500,000, per c.mm. or fall below 1,000,000. The oligocythæmia is usually greater in this than in myeloid leukæmia, but the ratio of whites to reds is not so much disturbed, the average ratio being 1 to 50, but it may stand at 1 to 3. The erythrocytes reveal changes in shape and staining reactions which are common to all severe anæmias.

Megaloblasts are occasionally present in small numbers.

Normoblasts are common in acute cases, less so in chronic. They outnumber the megaloblasts, but never appear in such numbers as they do in myeloid leukæmia.

Hæmoglobin.—This is invariably decreased and falls independently of the erythrocyte count, so that the colour index is always low, averaging about 0·5. In very acute cases the percentage may be as low as 10, or even lower, so that it may be very difficult to obtain a correct estimate.

Blood platelets.—These are said to be increased.

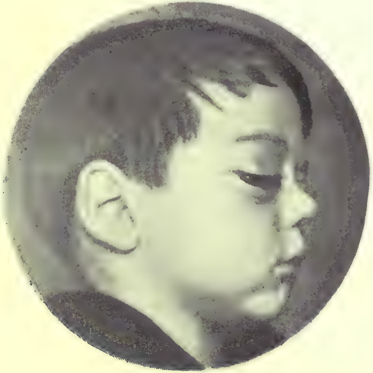
Treatment of Lymphoid Leukæmia

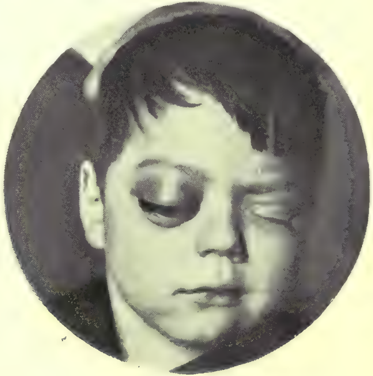
The treatment of acute lymphoid leukæmia is unsatisfactory, all that can be done is to relieve symptoms as they arise and as far as one can. There are no known measures capable of hindering its progress or curing the disease. In the chronic form surgical interference may be necessary for the relief of pressure by enlarged glands. Iodine used externally and internally has been found useful. Arsenic acts beneficially at first, but fails in relapses. It may be given according to regulations previously suggested. Bone-marrow does good occasionally, other organic extracts have failed. More recently X-ray therapy has been found beneficial in some cases. The rays should be projected over the enlarged glands, bones or spleen.

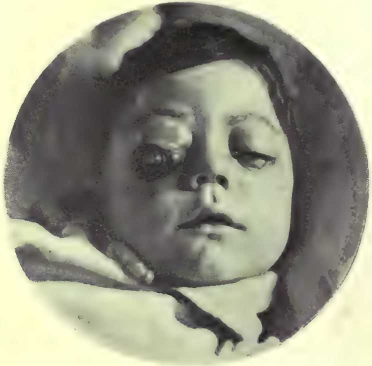
CHLOROMA

This rare disease, described under the names chloroma, chlorolymphoma and green cancer, is in many ways related to acute lymphoid leukæmia. It generally occurs in early life and in males more often than females. Nothing certain is known of its etiology. It is a disease of the bone-marrow associated with neoplastic depositions in various parts of the body connected with the bones and lymphatic glands, but metastises may occur in the viscera and elsewhere. In addition to these is a characteristic leukæmic blood state. The disease is so named from the peculiar green colour of the marrow and tumour formations, but a similar green colour of the marrow has occasionally been recorded in cases of acute lymphoid leukæmia. In association with the pallor of anæmia, one of the earliest symptoms is exophthalmos due to tumour formation in the orbit, accompanied by severe pain, and later by swelling of the frontotemporal regions. The masses may be palpable through the eyelids. The orbital swellings are accompanied by changes of vision, even to blindness, and fixation of the eyeball. In close relation with the temporal enlargement are associated deafness and vertigo, and facial paralysis. Swelling of the occipital regions may also take place. Pain is a more or less constant symptom; it may be severe, and

PLATE XVIII.







not only in the head but in the long bones and sternum. Orbital and temporal swellings are occasionally absent. The lymphatic glands may be found enlarged, and in some cases this may appear before the more characteristic facial enlargement; in other cases there may be little or no glandular affection. Splenomegaly is usually present, but not invariably so. Enlargement of the liver may also take place. The skin assumes a waxy pallor, petechiæ may occur, as may hæmorrhages into the mucous membranes, retinæ and elsewhere. The temperature usually runs an irregular febrile course. Emaciation and asthenia are very marked; coma usually precedes death. The growths on section present a peculiar green colour, which varies from a bright green to the dull colour of boiled dried green peas. It generally fades on exposure to the air, but may be reproduced on the application of peroxide of hydrogen. The usual preservative and fixing solutions destroy it. It is regarded as a fatty material of the nature of a lipochrome. This green colour is often present in the urine when first passed, but it gradually fades. Tumour formations may be found in the marrow in addition to the general reversion to the lymphoid type; they are usually present in the cranial and facial bones growing from the marrow, spreading beneath the periosteum and even into the dura mater; the ribs, sternum, spinal column, shoulder, pelvic or long bones may be affected. Deposits and tumour formations take place in the superficial and deep lymphatic glands and the ductless glands also. Any of the internal organs may become the seat of green tumour infiltrations and depositions. The lymphadenoid tissues throughout the body are all liable to become affected. The structure of the growths is that of a lymphoma, consisting of lymphoid cells held together by a fine reticulum. The green colour is due to the presence of strongly refractile green granules situated in the cells. It may fade rapidly on exposure to the air, or become a dirty grayish colour. The colour may be reproduced by the application of peroxide of hydrogen or ammonia. The pigment is "blued" by old tincture of guaiacum, and imparts this colour to the solution. It is soluble in absolute alcohol and ether, and stained by osmic acid. It does not give the iron reaction. In some cases the blood resembles ordinary myeloid leukæmia, but in the majority of cases it presents a mixed or lymphoid picture.

The following case, which came under my care, presented the usual classic features of the disease.

A. C. æt., 4½ years, had been perfectly healthy until July, 1907, when he became pale, tired and disinclined for any exertion. The pallor increased without any other symptoms until, at the end of October, he developed right-sided facial paralysis, and a few days later exophthalmos was first noted on the same side. Pain in the orbit, pain and deafness of the right ear followed, and later in the left ear also. The boy also complained of loss of power in the left leg, and that the left arm felt "tired and heavy." There was no vomiting, staggering gait, or optic neuritis. There was no splenic enlargement, but a few glands could be palpated in the right axilla. During the course of the next month his condition became worse, and he became semi-comatose. The right eye protruded and became chemosed, the left eye had commenced to protrude. Vomiting was frequent. The skin and mucous membranes were of a waxy colour. The temperature showed an evening rise to 100° F. or 101° F. The urine was green when passed. Exophthalmos gradually progressed until the right eye became completely disorganised and ulcerated. Swelling and bulging of both fronto-temporal regions took place. No secondary deposits could be discovered. The disease rapidly progressed, and death took place two months after the exophthalmos had been first noticed. The blood picture was characteristic. The number of leucocytes varied from time to time, the lowest count being 26,250, and the highest 252,000 per c.mm. a few days later.

The Blood in Chloroma

The main feature of the blood picture is the great, absolute and relative increase of large **mononuclear lymphoid cells,** which resembles acute lymphoid leukæmia. In this case they averaged 87 per cent. In the early stages **neutrophile myelocytes** were present, but were gradually displaced by **lymphoid cells.** These are large cells about three to five times the size of an erythrocyte. They possess a large, spherical, or somewhat indented nucleus, which responds feebly to nuclear stains, especially the triacid mixture; in the nucleus are two or three vesicular-looking nucleoli. Many nuclei appear free and devoid of surrounding protoplasm, while others are encircled by an extremely narrow rim of finely reticulated protoplasm responding rather feebly to basic dyes. With Leishman's stain the nucleus is coloured ruby-purple, the cytoplasm bluish-grey. With the triacid mixture it is difficult to see the cells at all, they stain so feebly. Other cells possess more cytoplasm, and the nucleus becomes indented on one side, in the bay of which, with Leishman's stain, pink, with the triacid violet granulation is seen to be gradually forming. Thus a definite transition can be made out from the lymphoid cell to the neutrophile myelocyte. In the later stages of the disease practically all the cells were lymphoid. In intermediate cells, the basophile cytoplasm appears stretched out around the neutrophile material, which arises in the neighbourhood of the archoplasm. The limiting layer gradually disappears and the cell becomes a neutrophile myelocyte. These lymphoid cells can be easily differentiated from the hæmal or small lymphocyte not only by their size but by the nucleus of the latter, staining a dark violet with Leishman's, a deep greenish-blue with the triacid solutions. The small lymphocytes are relatively diminished in number, but they may be absolutely increased.

Polymorphonuclear neutrophiles were both absolutely and relatively diminished, and many of them were quite free from granulation.

Eosinophile cells are usually present in the early stages; towards the close of my case they quite disappeared.

I found no basophile cells in the blood.

Erythrocytes are always very much diminished in number, the highest record in my case was 1,419,750, and the lowest 257,500 per c.mm. three days before death. The erythrocytes vary much in size and poikilocytes are present. Some exhibit polychromasia, but I have not seen any with basophile stippling. A few megaloblasts and normoblasts are usually present.

The **bone-marrow** in my case revealed an almost complete reversion to the lymphoid type, the cells being identical in appearance and staining reactions with those of the blood. The lymphoid elements were so general as to crowd out the neutrophile myelocytes which were few in number. Eosinophile myelocytes were scanty, thus contrasting with their prevalence in myeloid leukæmia. There was a fair number of large, richly granular basophile cells. Polymorphonuclear neutrophiles were scanty owing to the excess of lymphoid change; erythrocytes and erythroblasts were reduced to a minimum, a condition which accounts for the paucity of these elements in the blood. The marrow exhibited the characteristic green colour in the long bones, sternum, vertebræ and ribs, it was not so vivid as in the neoplasms. The general cell structure of the marrow and growths was alike, namely lymphoid. In films made from the marrow were numerous large cells, resembling myeloplaxes, with a spherical, oval, or twisted nucleus. The cytoplasm of these cells was clear and contained, in some cells a few, in others very many large granules of a peculiar greenish-grey colour. These granules were of varied size and shape, from the size of an eosinophile granule to an erythrocyte. Some were spherical, others cubical, and others occurred as irregular large masses. They did not stain by the methylene blue and iodine method, they were not basophile; with old tincture of guaiacum they turned blue, and with peroxide of hydrogen they exhibited a refractile green colour. None of these cells were found in the growths, the green material probably having been removed by fixative methods. These greenish deposits in the large marrow cells were really the material which gave it and the growths also the peculiar colour.

The autopsy revealed both orbital fossæ filled with firm uniformly green growth depressing the eye downwards and forwards, and supplanting the orbital fat. The growth

PLATE XIX.

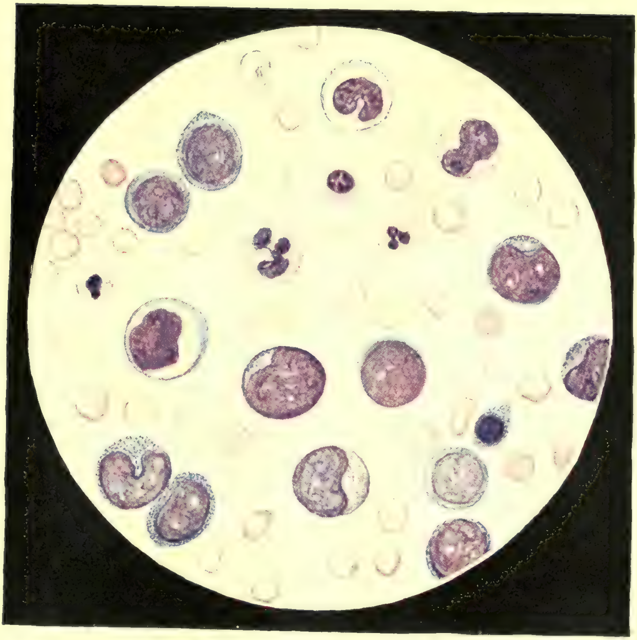


FIG. 1.

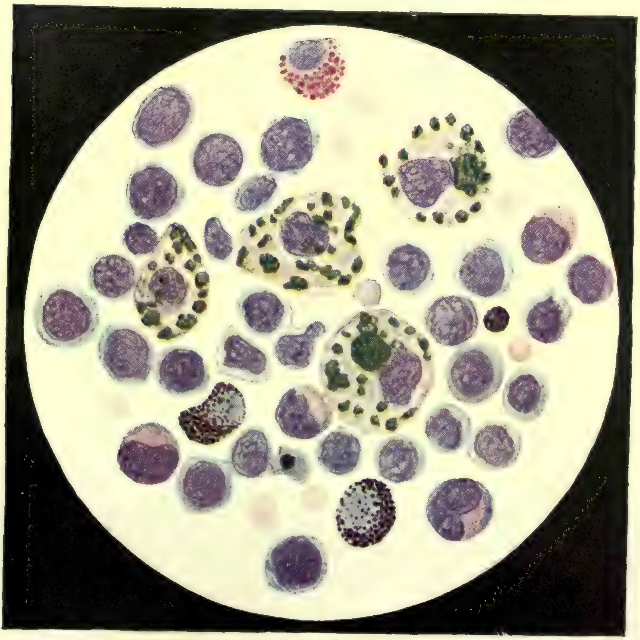


FIG. 2.

was easily separable from the orbital plates, it extended through the bones and infiltrated the temporal muscles on each side. In the cranium were found three small growths, two situated on the seventh and eighth nerves respectively of the right side, accounting for the facial paralysis and deafness, and another on the tentorium cerebelli on the left side. The cerebrospinal fluid was of a distinct pale green colour. The mediastinal, bronchial and lumbar glands were all affected; there was a small mass on the inner surface of the manubrium sterni. Another small green nodule was present in the left kidney. All the organs and tissues were anæmic, and the liver was a pale yellow colour, with a green tinge of the centres of the lobules. Microscopical examination revealed advanced fatty change with chloromatous infiltration round the portal and hepatic vessels. Numerous subserous petechial hæmorrhages were present over the lungs, in the pericardium, and acute purpuric hæmorrhages were present in the mucous membrane of the stomach. The growths consisted of masses of the usual "sarcoma-like" or lymphoid cells with round nuclei and clear staining protoplasm (in fixed and hardened sections). Sections of lymphatic gland showed infiltration with the growth, the cells being larger than lymphocytes.

Treatment

Treatment can only be exercised for the relief of symptoms. The disease always ends fatally.

LEUKANÆMIA

This composite term has been used to denote, as it implies, a condition which clinically and hæmatologically combines pernicious anæmia and leukæmia.

Symptoms

The general symptomatology of the disease conforms on the one hand to anæmia and leukæmia, there being asthenia, dyspnœa, pallor, retained appetite and nutrition, vomiting, hæmic and cephalic murmurs, retinal implication, hæmorrhages in and from various situations, œdema, irregular pyrexia, delirium, drowsiness and, later, stupor and coma preceding death. Three characteristic signs are almost constant; splenomegaly of varying degree, a progressive anæmia of the pernicious type, qualitative changes in the cytology of the blood, chief of which is the great excess of erythroblasts and the admixture of a myeloid mixed or lymphoid leukæmia. The lymphatic glands may or may not be enlarged.

The Blood in Leukanæmia

In reference to the anæmia the erythrocyte loss is usually profound and the colour index above normal averaging 1·5. The **erythrocytes** present all the peculiar deviations from the normal which are found in pernicious anæmia. There is, however, a great increase in the number of erythroblasts. The megaloblasts may or may not outnumber the normoblasts, the proportion varying from time to time. The disease may commence or first be recognised as pernicious anæmia, the leucocyte count not showing any increase, but a differential count of stained films will probably reveal the presence of neutrophile myelocytes. On the other hand, the leukæmic blood picture may be pronounced throughout with cellular excess; or if slight at first it may rapidly and unexpectedly towards the close of the disease become very marked. It may remain of the ordinary myeloid type throughout, become mixed-celled or revert to the lymphoid condition as seen in acute lymphoid leukæmia. The **polymorphonuclear neutrophile** cells may be relatively diminished, though absolutely increased. **Neutrophile myelocytes** or **lymphoid** cells may be in relative and absolute excess, and all stages of transition from non-granular to granular cells be seen, between the lymphoid cell to the neutrophile myelocyte. **Eosinophile cells** are not in evidence and basophiles are rare. The **bone-marrow** presents characters common to the anæmia and leukæmia corresponding to the blood picture. It may contain a marked excess of erythroblasts, and the same condition

PLATE XX.

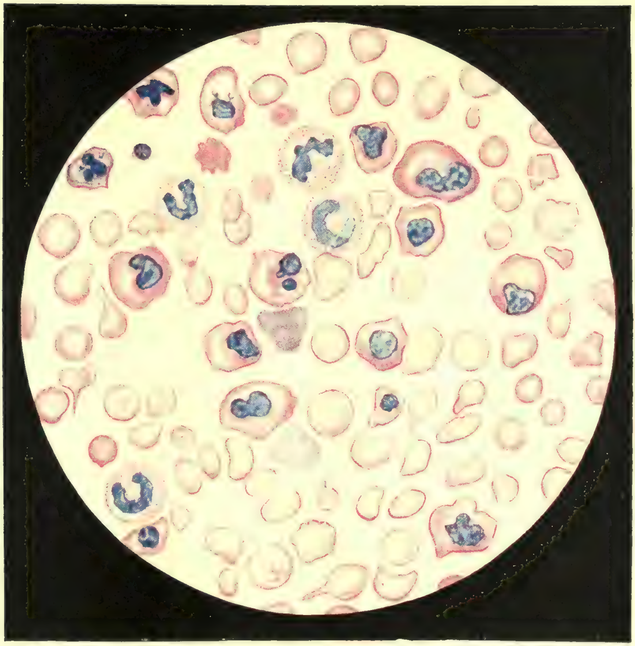


FIG. 1.

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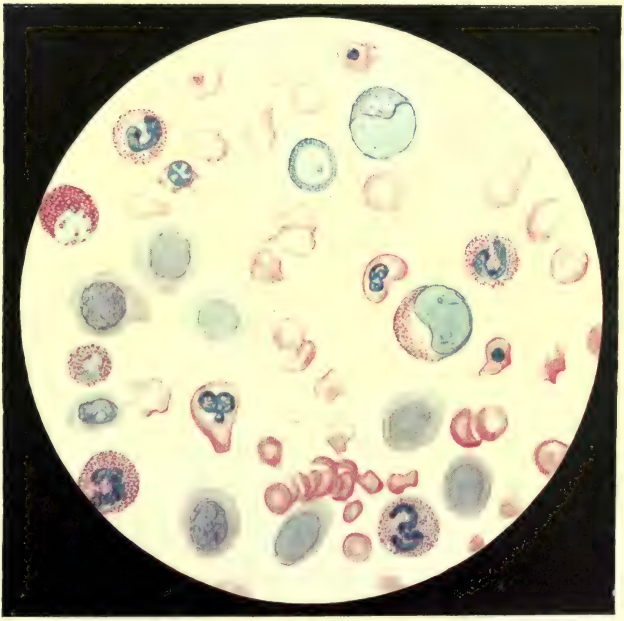

FIG. 2.

RJMBuchanan

may be present in the spleen and lymphatic glands. As a rule there is no deposit of iron in the liver.

Treatment

The course of the disease is rapid and no treatment seems to be of any avail to retard its progress or prevent the inevitably fatal issue.

LYMPHADENOMA (HODGKIN'S DISEASE)

This disease is characterized by enlargement of the lymphatic glands, generally chronic, with no tendency to suppuration; nodular formations in the viscera, occasionally in the skin; enlargement of the spleen and anæmia. Thus it resembles clinically lymphoid leukæmia, and the diagnosis between them depends upon the blood examination, and the fact that in lymphadenoma it is quite negative and free from any sign of leukæmia. The anæmia in the early stages is slight, but later it may be profound. The **erythrocyte** count corresponds. The percentage of **hæmoglobin** falls and is relatively low to the number of erythrocytes, so that the colour index is subnormal. When the anæmia is extreme normoblasts may appear, but are always scanty, and megaloblasts are extremely rare; alterations in size, shape, and staining reaction may also be present.

The **leucocytes,** as a rule, present no departure from normal, either in total number or relative percentage. Occasionally a relative increase of lymphocytes is present, with a corresponding relative decrease of polymorphonuclear neutrophiles, but without any total increase. Secondary complications produce the ordinary type of leucocytosis. Not only is there no leucocytic increase, but, on the other hand, in cases with marked anæmia there is often leucopœnia.

As in other grave anæmias, a few **myelocytes** may be found. **Eosinophiles** are scarce, and the **basophile** cells unaltered.

The **blood platelets** are said to be diminished. Although the blood picture affords valuable information as negative evidence of lymphoid leukæmia, it does not help in distinguishing lymphadenoma from other glandular enlargements, such as tuberculous and syphilitic adenitis, lymphomata or neoplasms of the lymphatic tissues.

CHAPTER XI

BLOOD DISORDERS IN INFANCY AND CHILDHOOD—THE BLOOD IN INFANCY—BLOOD DISORDERS—CLASSIFICATIONS—CONGENITAL MALFORMATIONS—CONGENITAL ANÆMIAS — ERYTHRÆMIA—PRIMARY ANÆMIAS—CHLOROSIS—PERNICIOUS ANÆMIA—SECONDARY ANÆMIA —ANÆMIA WITH SPLENOMEGALY—SPLENIC ANÆMIA OF INFANTS—MYELOID LEUKÆMIA—LYMPHOID LEUKÆMIA—MIXED LEUKÆMIA — OTHER CONDITIONS ASSOCIATED WITH ENLARGED SPLEEN—SYPHILIS—RICKETS—TUBERCULOSIS—GASTRO-ENTERITIS.

THE BLOOD IN INFANCY

IN the embryo the erythrocytes are developed partly from the blood vessels and partly from the liver. At a later period of intra-uterine life the liver, spleen and bone-marrow are all associated in blood formation, the change taking place about the fourth month, while towards the end of the gestation period, the liver and spleen fall out of the category, and the marrow assumes the sole birthplace of erythrocytes, which it retains after birth. It is, however, believed that, in early life at least, when special calls are made upon the marrow for blood formation, maybe in excess of its potentialities, the liver and spleen may resume their rôle to a certain extent and come to the assistance of the bone-marrow. In the blood of the prematurely born the three main characteristics are the preponderance of normoblasts, mononuclear leucocytes and the large amount of hæmoglobin. The large lymphocytes are prevalent before the seventh month, after which the small variety increase in numbers and the large decrease. Eosinophile cells reach a maximum at this time and become fewer as time goes on.

At birth the **erythrocyte** count is high, averaging 6,000,000 per c.mm. During the first twenty-four hours they increase numerically to between 7,000,000 and 8,000,000, and then fall to 5,000,000 in the course of ten days. The rise is due to a diminution in the amount of tissue fluids and coincident body weight, the subsequent fall depending upon fluid intake by the first feedings. The red count is always higher during fasting periods.

The individual erythrocytes vary very much in size, shape and staining reaction. Many are smaller than in the adult, others larger ; and on the whole they are more vulnerable. Erythroblasts are found in the blood at birth, though they are scanty, and in three or four days they disappear. Their presence in the blood at the end of six months must be regarded as pathological. Of course in premature children they are always more numerous. The amount of hæmoglobin at birth depends upon the richness of the blood in erythrocytes, it averages 104 to 110 per cent. This is a continuation of the abundance of hæmoglobin in fœtal life. During the first week, as the erythrocytes decrease, the hæmoglobin does the same, the two falling together ; during the first two weeks after this the colouring matter falls below the red cells, so that at the close of the third week there may be only 55 to 60 per cent. and the colour index low. This low colour index is normal in childhood for a long time, 70 per cent. being the average from the sixth month to the second year, after which it gradually rises, to reach the adult standard about the sixth or seventh year. The deficiency in hæmoglobin is supposed to be due to insufficiency of iron in the diet, or it may be a natural arrangement for the hæmoglobin to be spread over a normal relative excess of erythrocytes to allow increased area for oxygenation. An attempt to reproduce this is seen in adult anæmias by the breaking up of the erythrocytes and variations in shape and size.

The **leucocytes** average 20,000 per c.mm. at birth. By the end of the first week they stand at about 10,000 ; gradually rise to 15,000 by the sixth month ; then slowly fall to 10,000 by the end of the third year, and reach adult numbers by the sixth or seventh year. The first fall is due to a rapid decrease of the polymorphonuclears, which from the end of the first week to the sixth year remain practically stationary; the rise is produced by an influx of lymphocytes, and the normal infantile count depends upon their absolute and relative excess throughout the ensuing six years, when the adult number and relative percentages are reached. It will be seen that the blood contains an excess of non-granular cells, and this may depend upon the activity of adenoid tissues during early life, and for a short time after birth to a special activity of digestive leucocytosis.

Leucocytosis is associated with an increase in the polymorphonuclear cells, both physiological and also in infective or suppurative processes. It is an important fact, however, that in diseases which affect the blood-forming tissues there is a reversion to the infantile or antenatal condition and any coincident leucocytosis is lymphocytic in character.

The following table [1] gives the differential percentages of leucocytes in infancy as compared with adults:—

LEUCOCYTES.	INFANTS.	ADULTS.
	per cent.	per cent.
Small lymphocytes . .	50 to 70	20 to 30
Large lymphocytes and transitionals . . .	6 to 14	4 to 8
Polymorphonuclear neutrophiles	28 to 40	60 to 75
Eosinophiles	0·5 to 10	0·5 to 5

BLOOD DISORDERS IN INFANCY

When we consider the peculiarities of the blood in infants and children, it becomes evident that there is a stage of blood development still in progress during this period ere the adult condition is reached. During this stage the principal features are polymorphism of erythrocytes and leucocytes, and a richness in hæmoglobin in early infancy, followed by a deficiency with a low colour index in the later years. Further, there is a marked tendency to lymphocytosis, which may be aggravated on slight provocation, this being associated with adenoid activity. The blood formation, or growth of blood formative elements, may be retarded or easily disturbed; it is only infantile at any time and is thus far unformed, so that slight deviations from health may produce changes in the blood which come very close in their character to those changes which in the adult are associated with well-defined clinical diseases. The great tendency of the immature blood of the infant and young child is to revert to the fœtal character on comparatively slight disturbance. Although in healthy children the natural course is for the blood to grow towards adult type, its growth may be delayed, so that the blood of a child of three or four years or even more may be no further advanced than at one year. So that in making examinations, not only should the normal characters of infantile blood be kept in mind, but allowance must be made for development of the child at the time, if reliable interpretations are to be made. The difficulties of drawing correct conclusions from the examination of the blood in young children are many, and the pictures revealed by the microscope may be such that at one time, with slight departure from health, the appearances may be those of severe change as compared with an adult, so that an unfavourable prognostic impression arises, pointing to a fatal termination, which proves entirely false; while on the other hand, slight alterations may be present denoting a speedy recovery, which does not take place. Again, the variations and polymorphism which is so characteristic of infantile and child blood pictures, appearing in association with different diseases, makes it extremely difficult to classify the diseases of the blood in children in such a way as to render them free from criticism. The blood changes may resemble each other so closely that they seem to overlap.

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[1] Gundobin, *Jahrb. f. Hinderheilk*, 1893, vol. xxxv, p. 187.

The blood changes which are more or less common to all the anæmias of infancy and childhood are: (1) A relative deficiency of hæmoglobin, with a low colour index; (2) the polymorphism of the erythrocytes and occurrence of erythroblasts; (3) the tendency to leucocytosis, often lymphocytosis, with frequent occurrence of myelocytes.

CLASSIFICATION OF BLOOD DISORDERS OF INFANCY AND CHILDHOOD

In addition to the characters of the blood changes mentioned above, it is a notable fact that many of these, and especially the anæmias, are associated with enlargement of the spleen ; and attempts have been made to classify the anæmias into those with and those without splenic enlargement, but the classification is not satisfactory, from the fact that anæmia does occur without it and splenomegaly without anæmia. With the splenic enlargement and anæmia it was thought that erythroblasts were more numerous, but this is not necessarily the case. The number of erythroblasts is not in direct relation with the splenomegaly.

Monti and Berggrün[1] divided the anæmias into mild and grave, and these subdivided into those with and those without leucocytosis—

- Secondary anæmia of infancy
  - Mild
    - With leucocytosis.
    - Without leucocytosis.
  - Grave
    - With leucocytosis.
    - Without leucocytosis.

A modification of this classification has been suggested by Morse[2]—

- Primary anæmia
  - Pernicious anæmia.
  - Leukæmia.
- Secondary anæmia
  - Mild anæmia.
  - Mild anæmia, with leucocytosis.
  - Severe anæmia.
  - Severe anæmia, with leucocytosis.

A more elaborate classification, based largely upon the causes of the anæmias, was arranged by Monti,[3] in 1894. These are secondary anæmias, the primary remaining separate—

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[1] *Die Chronische Anämie im Kindesalter,* Leipzig, 1892.

[2] *Archives of Pediatrics,* 1898, vol. xv.

[3] *Wiener, Med. Woch.,* 1894.

1. Congenital, due to	Syphilis Tuberculosis Malaria, etc.	In the mother during pregnancy.
2. Acquired	(1) Hæmorrhage	From the navel. After circumcision. Scurvy, purpura, hæmophilia, Werlhof's disease, melæna.
	(2) Other causes	Inanition. Bad hygiene. Post-febrile. Nephritis. Diarrhœa. Serous effusions. Syphilis. Rickets. Suppuration. Diseases of liver, spleen, bone, or lymphatic glands.

R. Hutchison[1] bases his classification on both clinical and pathological data, as follows—

1. Congenital abnormalities of the blood	Malformation of the blood as a tissue	Cretinism. Infantilism. Mongolism.
	Abnormalities in the total quantity of blood or of some of its cellular constituents	Congential anæmias.
	Abnormalities in chemical composition	
2. Primary anæmias	Chlorosis. Pernicious anæmia.	

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[1] Goulstonian Lectures on "Some Disorders of the Blood and Blood-forming Organs in Early life," *Lancet*, vol. i, 1904.

3. Secondary anæmias	Malnutrition. Infective processes. Infantile scurvy. Oral sepsis. Syphilis. Tuberculosis. Chronic diarrhœa. Suppuration.
4. Blood disorders associated with enlargement of the spleen	Splenic anæmia of infancy.
5. Leukæmia	Myelogenous. Mixed. Lymphatic.
Changes in the thymus	Status lymphaticus.
6. Affections of adenoid tissues	Tuberculous adenitis. Hodgkin's disease. Lymphosarcoma.

CONGENITAL MALFORMATIONS OF THE BLOOD

In reference to these, in cretinism and mongolism there does not seem to be any special departure from the normal, or the blood may show similar changes to those of secondary anæmia. In infantilism, the blood retains its infant character, and the more delicate and feeble the child the longer the change to adult characters is postponed. The blood denotes an unusual prolongation of the period of activity of the adenoid tissues. The leucocytes may remain normal, or there may be an excess of non-granular cells.

CONGENITAL ANÆMIAS

In the fœtus the blood-forming organs are independent of the mother relative to the cellular elements of the blood. Cases of anæmia occur in the new-born and early infancy apart from any association with the health of the mother, bad feeding, diarrhœa, vomiting, syphilis, etc., which so often are causative factors. Their true nature is not definitely settled. In the liver of the fœtus and new-born there is a store of iron deposited ready to be drawn upon after birth. It may be that this is deficient; or there may be a congenital aplasia of the bone-marrow, which renders it an inefficient agent in blood formation.

The anæmia is sometimes associated with marked icterus neonatorum expressive of blood destruction beyond the immediate regenerative capabilities of the bone-marrow. The blood itself may show signs of aplasic anæmia.

CONGENITAL ERYTHRÆMIA

Contrasting with the congenital anæmias is the erythræmia associated with congenital cyanosis and hyperplasia or excessive activity of the bone-marrow and thymus. In this condition there is an excess of erythrocytes and hæmoglobin in the blood. In the thymus there is a persistence of intra-capillary blood formation.

PRIMARY ANÆMIAS

Chlorosis

If chlorosis be regarded as a clinical entity peculiar to young women, then it cannot be supposed that such a condition should arise in early childhood, and some authorities consider that chlorosis per se never occurs in infants and young children. It is not uncommon, however, to find blood states in children, although associated with rickets or other allied conditions which resemble chlorosis clinically and hæmatologically. The blood of children is relatively poor in hæmoglobin and any cause which hinders the formation of hæmoglobin tends to produce a chlorotic state of the blood. This is especially so when iron in the food is deficient, and as the natural food of infants—milk—is poor in iron, so children who are fed too long upon it become chlorotic, when the hepatic store has come to an end. It may justly be assumed that chlorosis and rickets are the dual expression of food poverty, not that the chlorotic state is a sequel to the latter or dependent upon it. Such a condition rapidly disappears with a diet rich in iron, or on the administration of iron medicinally.

Pernicious Anæmia

If we confine the definition of pernicious anæmia as one for which no cause is known, then we must regard it as excessively rare in children, and comparable thus far with the disease in adults. The fact that in secondary anæmias of infancy and early childhood, the blood pictures may be identical with those found in the pernicious anæmias of adults leads to the idea that we are not quite correct in classifying the latter as purely primary, but rather that all such are really secondary, although the cause may not be definitely known. The blood picture so characteristic of the disease in adults, if found in the anæmia of infancy, does not help to any material extent to classify the latter as pernicious in type, for any grave anæmia of secondary origin may exhibit identical changes with the appearance of normoblasts and megaloblasts, megalocytes and polymorphism of the red cells. The relative lymphocytosis which occurs in adults, and also in children, does not convey the same significance in the latter, because it is a common condition in any anæmia of early life. The leucocyte count cannot be directly compared in adults and children, because in the latter the average is always greater.

The post mortem findings in the bone-marrow, revealing as they do in the adult a change from yellow to red marrow with total increase, are without value, for the same appearances are normal to the child.

If in the adult the condition be one of hæmolysis due to the absorption of some or other poison, then one would expect its common occurrence in children because of the vulnerability of the blood at their early age. It has been suggested that the disease in adults may be a form of secondary anæmia due to some cause the nature of which is unknown; for in some cases the cause is known, e.g. bothriocephalus anæmia, the removal of which is followed by recovery of the blood. In children grave secondary anæmia may be produced by toxic absorption, and oral sepsis is often associated with it, just as it is in the pernicious anæmia of adults. If, again, the adult form be due to a reversion to a fœtal condition of the blood-forming organs, with a characteristic blood picture, one would expect to find pernicious anæmia often in young children, whose hæmogenesis is not far removed from the fœtal stage.

One distinguishing feature between the grave anæmias of children and the pernicious anæmia of adults is the fact that, in the latter there is a deposit of iron in the viscera, especially the liver, which is not present in the former. It is doubtful if much stress should be laid upon this, because in the adult it may represent a more complete reversion to the fœtal state, in which there is a normal hepatic store of iron. It may be, that if cases of adult pernicious anæmia died in the early stages, this iron deposition would be found slight or absent; and that its amount depends upon the length of time this disease has been in progress. On the other hand, in the child, death may come so early, that no deposition has had time to take place; the fœtal store has been used up, and the food lacking iron, as it does for children (keeping down the hæmoglobin percentage and colour index), does not provide a sufficiency for the blood, so that no deposit in the liver is to be expected.

In children recovery takes place from secondary anæmias which to all intents and purposes resemble adult pernicious anæmia, because the natural progress of the blood-forming tissue is towards growth and blood formation, sufficient for and equal to the demands made upon it; while in the adult the infantile or fœtal reversion in the bone-marrow, with its characteristic blood picture, is totally inadequate to cope with the needs of the adult human being. Thus when from whatever cause the blood and blood-forming organ of the adult is so exhausted as to resemble that of a young child, the demands made are so overwhelming and the response so feeble that the exhaustion becomes complete and death ensues, and the anæmia is endowed with a special terminology, it is called "pernicious." The same anæmia in the child, because recovered from, escapes this designation as a special and definite entity different from all others. There is much reason to regard the two as identical, but with different results, because of disproportionate conditions.

SECONDARY ANÆMIA

Children are very susceptible to influences which are causative of secondary anæmia, in whichever way they may act, either by causing blood destruction—hæmolysis—or by interfering with and injuring the blood-forming organs in such a way as to hinder blood formation. The general tendency is for such anæmias to become severe, more so than in the adult, and when pronounced, the blood picture may be identical with that of pernicious anæmia in the adult. When slight the hæmoglobin alone may be reduced so that the blood state is chlorotic, but with severer grades, erythrocyte loss follows, nucleated red cells appear and marked poikilocytosis, with alterations in staining reaction. The reason for the changes in the blood are twofold. Deficiency in hæmoglobin is normal in the infant, and its formation slow, so that deleterious influences soon cause a further reduction. The polymorphism of the cellular elements is easily accounted for by the fact that the bone-marrow is at its maximum of hæmogenesis relative to the needs of the organism, hence extra calls upon it are not compensated by hyperplasia, but by increased rapidity of cell production, resulting in an influx of immature forms to the blood stream. This happens at an earlier period and from slighter causes the younger the child.

The leucocytes may remain normal for the age of the child, or an increase may take place, so that leucocytosis is a common occurrence in severe anæmias. A relative and absolute excess of lymphocytes occurs, especially in cases associated with gastro-intestinal irritation. A polymorphonuclear neutrophile leucocytosis is generally expressive of some complication.

The causes of secondary anæmia embrace all factors which affect nutrition and growth, as well as infective processes. Malnutrition, improper diet, too prolonged use of milk, deficiency of iron in the food, want of sunlight and fresh air, etc., are all responsible individually or severally ; acute rheumatism is a potent factor, resulting in prolonged hæmoglobin deficiency ; diphtheria, in which myelocytes appear in the blood, oral sepsis, syphilis, tubercle, acute and chronic diarrhœa, infantile scurvy, and many others, give rise to secondary anæmia of different grades of intensity.

ANÆMIAS WITH SPLENOMEGALY

Enlargement of the spleen is a common accompaniment of anæmias in children. In infancy the spleen is regarded as taking part in the formation of lymphocytes, a function which it shares with the thymus and other adenoid structures. In the fœtus it helps in the formation of erythrocytes, and it has been held that under special circumstances it may, after birth, take on a function similar to the bone-marrow and give rise to both erythrocytes and leucocytes. According to Wolff [1] the indifferent lymphoid cell of the spleen may compensate marrow deficiency by developing in this dual direction.

SPLENIC ANÆMIA OF INFANTS

In this category are included the conditions known as "Anæmia splenica infettiva die bambini" of the Italian writers, and the "Anæmia pseudo-leukæmia infantum, of von Jaksch.

The disease occurs in children between six months and two years of age. There is emaciation, waxy pallor, sometimes with a pale yellow tinge, a tendency for "bossing" of the cranial bones, enlargement of the liver and to a slight extent of the lymphatic glands. Enlargement of the spleen is a characteristic feature. Hæmorrhages and petechial eruptions may occur. There is a tendency to diarrhœa, bronchitis and broncho-pneumonia. Congenital syphilis and rickets bear some relation to the disease. In some instances it has arisen in several members of a family, as it has been seen to do in the adult form. There may be a family history of tubercle.

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[1] *Zeitschrift für klinische Med.* vol. xlv., 1902.

There is reason to believe that the diseases described under the different terms mentioned above are all practically the same, and may be included in the term "splenic anæmia of infants" used in this country. It is not universally agreed that the disease is a separate clinical entity. It has been regarded as a secondary anæmia with special characteristics associated with its occurrence in early life; as an aleukæmic stage of true leukæmia; as an intermediate stage between leukæmia and pernicious anæmia or a mixture of both. The opinion now usually held by most English writers is that it is a disease *sui generis.* There does not seem to be any constant or definite relation between this disease and rickets or congenital syphilis. Certainly the former often, the latter seldom is associated with it, not, however, as cause and effect, but rather that they are coincident. The anæmia with splenomegaly seen in congenital syphilis occurs at an earlier age than splenic anæmia. There is little ground for belief that splenic anæmia is ever transformed into a true leukæmia. As to its being an intermediate stage between pernicious anæmia and leukæmia there is not much to be said; neither the blood picture or post mortem findings help much, and myelogenous leukæmia is a rare condition in children. In support of the view that it is a disease *sui generis* is the blood picture, which presents a special character which distinguishes it from secondary anæmias. This is the polymorphic nature of the leucocytosis. There is nothing characteristic in the morbid anatomy of the spleen or other organs in this disease which may not occur in other anæmias.

It is on clinical evidence and the blood picture combined that this disease may be said to stand alone. It is important to point out that there is no direct relation between the degree of splenomegaly and the erythrocyte count, but there is between it and the amount of leucocytosis. The blood changes found are as follows—

The erythrocytes are always reduced, but never to an extreme degree, averaging from 2,000,000 to 3,000,000 per c.mm., rarely less. The hæmoglobin is reduced, but not much under the normal low percentage of children.

There may be a disproportion between erythrocyte and hæmoglobin loss. Erythroblasts are common, normoblasts plentiful but bearing no relation to the severity of erythropœnia; they outnumber the megaloblasts which appear in small numbers. There may be leucocytic decrease or increase; if the latter and polymorphonuclear some complication is indicated. Non-granular cells are usually responsible for the increase; large, small and intermediate varieties occur, and myelocytes with scanty granulation are usually present. Eosinophiles are not evidenced, but in most cases may be present in fair numbers.

It is this polymorphism in the leucocytes that imparts to the blood its distinctive character. The greater the erythopœnia and polymorphic leucocytosis, the more unfavourable the outlook.

What the true etiology of the disease is has not been determined. It has been suggested by Clive Riviere[1] that it may be due to the absorption of specific toxins from the alimentary tract. The relation, if any, between splenic anæmia of infants and adults remains unsettled. Leaving out the differences in their respective blood changes, one remarkable fact distinguishes them from one another, that in the disease of children recovery takes place in a fair proportion of cases, and it is of shorter duration, while in the adult it is very chronic and progresses to fatality.

The adult form is supposed to occur in rare instances in children, and cases have arisen during the intermediate periods of life which present difficulties in their true classification.

MYELOID LEUKÆMIA

This disease is extremely rare in infancy, but cases have been recorded from time to time, and even in the new-born.

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[1] *Trans. of Royal Med. and Chirurg. Soc.*, vol. lxxxvii, 1903.

It seems that many so-called cases of myeloid leukæmia cannot be with certainty regarded as such, either from the fact that the differential leucocyte counts have not been carried out in sufficient detail, or the more approved methods of preparation and staining were not used. It is strange that, in view of the ease with which marrow disturbance is invoked in children, and that myelocytes are so often found in other blood disorders, myeloid leukæmia as recognized in the adult should be so uncommon in early life. There are no special differences in the blood picture in either period.

LYMPHOID LEUKÆMIA

This is, practically speaking, as common in infancy and childhood as the previous variety is rare. Guinon and Jolly[1] classify them as conforming clinically to three types: (1) Those in which the anæmia is profound, with general glandular enlargement and hæmorrhagic tendencies in the later stages; (2) those in which there is a hæmorrhagic tendency from the outset, resembling purpura; (3) pseudo-scorbutic cases with lesions in the buccal cavity.

The blood picture is similar to that seen in adults, a reversion to the fœtal type of marrow cell, the lymphoid cell being the principal feature. Chronic lymphoid leukæmia, with a preponderance of small cells, is uncommon in children.

There seems to be some analogy in the marrow disturbance in children producing special blood change in anæmia and leukæmia on the erythrocyte and leucocyte branches. On the one hand there is a marked tendency for the blood to revert to the fœtal type, with resulting deformity of size, shape and staining reaction of the red cells and the appearance of erythroblasts of both varieties; this condition of things arising with slight disturbance and with little or no period of transitional change. On the leukæmic side, the immediate tendency is to the fœtal type, without any special intermediate condition represented by myeloid as distinct from lymphoid change, the occasions on which it occurs presenting the rare instances of myeloid leukæmia. The mixed celled leukæmias which may occur in children, presenting both neutrophile myelocytes and lymphoid cells, may also be regarded as intermediate forms. In the adult the intermediate stage between normal blood and "pernicious anæmia," in the form of "secondary anæmia," is present before the fœtal reversion is complete, and in reference to leukæmia an analogous intermediate stage or period is represented by chronic myeloid leukæmia.

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[1] *Revue Mensuelle des Maladies de l'enfance*, June, 1899.

BLOOD CHANGES OCCURRING IN INFANCY AND EARLY CHILDHOOD, ASSOCIATED WITH SPECIAL DISEASES AND ENLARGEMENT OF THE SPLEEN

Syphilis

In this disease, either congenital or acquired, anæmia, often severe, is commonly present. Enlargement of the spleen is present in variable degree, and the same may be said of the lymphatic glands. The red cells are diminished, and the hæmoglobin in lower proportion, so that the colour index is subnormal. Deformities in size and shape of the erythrocytes are present. A leucocytic increase takes place, mainly due to a relative lymphocytosis, the polymorphonuclears being decreased; myelocytes may appear.

Rickets

This condition is also associated in many cases with splenic enlargement. The erythrocyte loss is not usually so great as in syphilis, the loss of hæmoglobin resulting in a low colour index. Similar morphological changes are seen in the erythrocytes, and a leucocytic increase is generally due to lymphocytes.

Tuberculosis

Splenic enlargement may be associated with tuberculosis; the anæmia is due to erythrocyte and hæmoglobin loss, of variable degree, with a low colour index. There need not be any marked alteration in the number of leucocytes; if a mixed infection be present a polymorphonuclear leucocytosis generally occurs.

Gastro-enteritis

In cases associated with gastro-intestinal irritation, anæmia may assume most profound intensity. Enlargement of the spleen is often present. Erythrocytic loss may be great, hæmoglobin loss greater, with low colour index. Deformed and nucleated red cells appear. Lymphocytes are much in evidence, being responsible for the leucocytic increase, and occasionally myelocytes may be found. Some of the most profound anæmias I have met with have been in children suffering from "summer diarrhœa."

CHAPTER XII

SYNOPSIS OF CHANGES IN THE BLOOD ASSOCIATED WITH SPECIAL DISEASES

SYNOPSIS OF CHANGES IN THE BLOOD ASSOCIATED WITH SPECIAL DISEASES—ACUTE INFECTIOUS DISEASES—LOCAL AND GENERAL SEPTIC INFECTIONS—CONSTITUTIONAL DISEASES AND DUCTLESS GLANDS—MALIGNANT DISEASE—TUBERCULOSIS—SYPHILIS—DISEASES OF THE LUNGS AND PLEURÆ—DISEASES OF THE CIRCULATORY SYSTEM—DISEASES OF THE DIGESTIVE SYSTEM—DISEASES OF THE LIVER—DISEASES OF THE GENITO-URINARY SYSTEM—INTESTINAL PARASITES—DISEASES OF THE NERVOUS SYSTEM—THE HÆMORRHAGIC GROUP OF BLOOD DISEASES—BLOOD CHANGES DUE TO SEVERE BURNS—DISEASES OF THE SKIN—BLOOD CHANGES DUE TO THE ACTION OF CERTAIN POISONS.

ACUTE INFECTIOUS DISEASES

Fever in general.—The contraction of the peripheral vessels so common in fever and the loss of water may be accountable for the rise in erythrocyte count and specific gravity which takes place. This may, however, be balanced in some measure by hæmolysis.

During defervescence and proportionate to it the erythrocyte count falls, due partly to cell destruction, vascular dilation and reabsorption of fluids. Thus a certain degree of anæmia is established, which in some cases may be profound. Changes in the reaction of the blood are doubtful. Quantitative changes in leucocytes and fibrin formation are not constant. Leucocytosis is the rule, the neutrophiles being increased at the expense of others ; the eosinophiles may quite disappear, but with defervescence a fall in the leucocyte count takes place, the neutrophiles may be diminished, but the eosinophile cells generally increase in numbers, and the rise may last for a considerable time. The granules of many leucocytes sometimes respond feebly to stains, or may change their affinity for certain stains.

Acute Pneumonia.—The organism of this disease may be isolated from the blood in some cases, especially if secondary infections take place. Its presence in the blood stream adds to the gravity of the condition and prognosis. The blood as a rule coagulates rapidly, and the fibrin is increased. The erythrocyte count may be normal, in cyanotic cases increased. After the crisis both erythrocytes and hæmoglobin fall, the latter more so than the former, and its regeneration takes longer. The specific gravity of the blood is said to be raised during the febrile state, falling with the temperature. Erythrocytes may exhibit qualitative degeneration changes, and occasionally normoblasts or even megaloblasts may appear.

Leucocytosis is the rule and generally falls by lysis just before the critical fall of temperature. Should resolution be delayed or secondary complications arise, e.g. empyema, the leucocytosis continues. The leucocytosis is neutrophile ; after the crisis the eosinophiles increase. Myelocytes may occur in small numbers. Transitional and stimulation forms are not uncommon. Iodophilia is always present. According to Cabot there may be a mild infection, with vigorous resistance on the part of the patient with slight leucocytosis ; or moderate or severe infection with vigorous resistance and marked leucocytosis, but if a virulent infection and feeble resistance leucocytosis is absent. Except in very mild cases, an absence of leucocytosis is of grave import, but the presence of eosinophiles is favourable. In children with bronchopneumonia, the leucocytosis may be largely due to excess of lymphocytes, so that the blood may resemble an acute leukæmia. Blood platelets diminish during the fever, to be increased after the crisis.

Typhoid fever.—Eberth's bacillus is present in the blood and can be isolated in nearly every case. They are to be found as early as the second day of the illness,[1] they persist during the febrile period and disappear with its defervescence, and there is definite relation between the severity of the disease and the bacillæmia. The bacillæmia returns with a relapse. Bacilli are easily obtained from the rose spots, and may be found before the serum reaction. The specific gravity is raised with the fever.

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[1] Coleman and Buxton, *Amer. Journ. Med. Sci.*, June, 1907.

Anæmia always occurs, the erythrocyte loss taking place particularly in the second week; the hæmoglobin declines rapidly, in a greater degree than the cells, and its regeneration is slower than that of the erythrocytes. Anæmia is rapid if there be hæmorrhage, but excessive diarrhœa and the use of cold baths may give a higher count of erythrocytes than is usual. The more prolonged and severe the disease the greater the anæmia.[1]

Qualitative changes are often present in the erythrocytes proportionate to the degree of anæmia. It is of great diagnostic importance that typhoid fever is unaccompanied by a leucocytosis, in fact, leucopœnia is often present. There is an absolute diminution of neutrophiles, with a relative increase of lymphocytes. The eosinophiles are diminished or absent, but increase during convalescence, and this has been regarded as a favourable sign. According to Higley[2] the differential count is characteristic before the eruption appears.

The leucopœnia of typhoid is proportionate to the severity of the disease. Should leucocytosis occur, it points to some or other form of complication, e.g. perforation, unless there be severe general peritonitis, but it may occasionally be present without. Iodophilia is present. Blood platelets and fibrin are diminished.

Typhus fever.—A degree of secondary anæmia accompanies this disease and by some observers leucocytosis is said to be absent, or a leucopœnia may take place as in typhoid fever. Hæmoglobinæmia may be present in some cases. Love[3] gives a detailed examination of twenty-six cases, nine of which proved fatal and seventeen recovered. There was a definite leucocytosis averaging 24,000 per c.mm.

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[1] Thayer, *Johns Hopkins Hosp. Reports*, vol. viii, 1900.

[2] *Med. Rec.*, September 19, 1903.

[3] *Journ. Path. and Bact.*, April, 1905.

In fatal cases the leucocytosis appeared after the rash, increased to the crisis, and then declined if death did not follow immediately. No eosinophiles were found in fatal cases. In fatal cases there was a higher erythrocyte count than normal. In cases which recovered the leucocytosis reached a maximum shortly before the crisis and fell during convalescence. In non-fatal cases eosinophiles were never completely absent. Inflammatory complications produced secondary leucocytosis.

Diphtheria and tonsillitis.—Although the appearance of a person suffering from diphtheria may denote the presence of anæmia, the loss of erythrocytes and hæmoglobin may not be marked unless the infection be a severe one. Qualitative changes in the erythrocytes are not common; normoblasts may occasionally be found. The hæmoglobin regeneration takes place more slowly than the erythrocytes. It is a notable fact that in cases treated by antitoxin the anæmia is as a rule slight and rarely severe, a marked difference is shown in this as compared to those cases not so treated. Leucocytosis is the rule and is pronounced in severe cases; but when mild or extremely virulent it may be absent. The leucocytosis increases as the disease progresses, and falls during convalescence, but there does not seem to be any definite relation between it and the pyrexia, amount of membrane, or glandular enlargement. Antitoxin has no effect upon the leucocytosis. The neutrophiles are increased and the eosinophiles diminished, but a subsequent rise of the latter during convalescence takes place. Myelocytes are often present, and regarded as a grave prognostic sign, as they have usually been found in fatal cases by Engel.[1] Iodophilia is present.

In tonsillitis there may be anæmia in severe cases. Leucocytosis generally occurs.

Scarlet fever.—There is no great erythrocyte or hæmoglobin loss in this disease, but the hæmoglobin may fall considerably in severe cases, and hæmoglobinæmia is said to occur sometimes. Occasionally normoblasts have been found. Leucocytosis is the rule, and is usually greatest two or three days after the eruption appears. It is generally proportionate to the severity of the attack, and in severe cases may persist after the temperature has reached normal. Eosinophiles are present in all but the severest cases, and their absence is regarded as a bad prognostic sign. Transitional leucocytes may be increased, and myelocytes are occasionally seen. The leucocytosis of scarlet fever is an important diagnostic feature between it and measles, in which leucocytosis is absent. In severe cases coagulation is rapid and the fibrin increased. Blood platelets are unaltered, but increase during desquamation. The specific gravity is said not to change unless there be acute nephritis, when it falls.

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[1] *Deut. Med. Woch.*, 1897, vol. xxiii.

A. G. Banks[1] points out that the opsonic content, as tested to four strains of streptococci, is decreased during the early period and rises again with defervescence; in severe anginal cases it remains low. He considers a definite relationship to the streptococcus scarlatinæ obtains.

Measles.—There is little or no alteration in the erythrocytes or hæmoglobin in this disease. The peculiar feature is that the leucocyte count remains normal, or there may be leucopœnia, which generally is most marked at the height of the eruption, the normal returning as the eruption fades. During the incubation period a neutrophile leucocytosis is said to occur, disappearing with the eruption. Occasionally a relative increase of lymphocytes occurs. The eosinophiles are diminished or absent. Should complications of an inflammatory nature arise they may cause leucocytosis. Apart from complications, there is no increase of fibrin, and blood platelets are few. The blood condition in Rötheln is similar to that of measles.

Mumps.—There are no special blood changes unless complications invoke leucocytosis.

Pertussis.—There is no special change in erythrocyte or hæmoglobin content. There is leucocytosis, which is largely accounted for by an absolute and relative increase of the lymphocytes, which comes on early, even before the characteristic cough, and lasts long. It has been observed more particularly in young children, and has been attributed by Erlich to irritation of the tracheo-bronchial glands. It is regarded as important in diagnosis.

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[1] *Journ. Path. and Bact.*, October, 1907.

Variola.—Hayem considers that no other disease produces so great a destruction of erythrocytes. During the febrile stage the count remains normal or may be raised, but when the temperature finally falls a marked fall of erythrocytes takes place. Anæmia is particularly marked in hæmorrhagic cases and comes on more rapidly. Qualitative changes in the erythrocytes are present, with severe anæmia. Leucocytosis occurs, especially during the vesicular and suppurative stages of the eruption; it disappears during convalescence, but may return if complications arise. In virulent and hæmorrhagic cases there may be leucopœnia. The percentage of neutrophile cells is diminished, but the mononuclears are increased. Eosinophiles may be diminished. Myelocytes are present, particularly in hæmorrhagic cases; these and stimulation forms are said to be present from the beginning. Mast cells appear during the suppurative stage. Blood platelets are decreased. Fibrin is increased during the stage of pustulation.

In varioloid leucocytosis does not occur, unless there be suppuration. The increase is said to be due in some cases to the neutrophiles, in others to mononuclears.

Varicella.—The blood changes resemble that of variola, but are present in a much less degree.

Rheumatic fever.—In this disease anæmia is a constant feature, and is a reliable index of the condition of the patient. Fibrin formation is markedly increased, but the rate of coagulation slow. Lactic acid is said to be present in the blood and the alkalinity is lessened.

There is a rapid fall in the erythrocyte count and a still more rapid and intense fall in hæmoglobin. Qualitative changes take place in the erythrocytes, and normoblasts may appear.

Leucocytosis is present and proportionate to the severity of the attack. Complications and joint implications increase it. The eosinophiles do not disappear during the fever, and may rise above normal during convalescence. Myelocytes and stimulation forms may occur. Blood platelets are increased.

Erysipelas.—Moderate anæmia may occur in severe cases, the hæmoglobin suffering most. Qualitative degenerative changes may be present in the erythrocytes. Fibrin and blood platelets are increased, and alkalinity said to be lessened. A neutrophile leucocytosis is present in well-marked cases and when the local condition is spreading; it depends upon the severity of the infection, and falls both before defervescence and with it. Myelocytes and stimulation forms may appear.

Influenza.—A moderate anæmia may arise. Leucocytosis is slight or absent and this disease is regarded as one of the acute infections without leucocytosis.

Asiatic cholera.—The alkalinity of the blood is diminished, so that by some it is regarded as having an acid reaction. The specific gravity is raised. Marked polycythæmia occurs in the algide stage and the hæmoglobin content is raised. Marked neutrophile leucocytosis occurs in bad cases, and its presence is regarded by Rogers as an unfavourable sign and denotes fatality.[1] According to Sherrington [2] the mast cells are increased.

Glanders.—The bacilli have been found in the blood in this disease. Leucocytosis is usually present.

Bubonic plague.—The *bacillus pestis bubonicæ* is present in the blood and may be isolated from it. There is a certain degree of polycythæmia, the erythrocyte count being raised. There is a marked neutrophile leucocytosis and eosinophile loss. Blood platelets are diminished and coagulation much retarded.

Actinomycosis.—Anæmia of varying severity occurs with more marked loss of hæmoglobin than erythrocytes. Leucocytosis is usual.

Tetanus.—There may be a slight leucocytosis.

Beri-beri.—There is a slight degree of anæmia. The leucocytes are not increased, but eosinophilia is said to be marked in the acute stages.

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[1] *Lancet*, September 6, 1902.

[2] *Proc. Roy. Soc.*, 1894, p. 189.

Relapsing fever.—The spirilli are present in the blood. Anæmia occurs during the paroxysms with recovery in the intervals. Serum reaction is positive as demonstrated by Lowenthal.[1] Leucocytosis is present, and melanin has been found in the leucocytes and free in the plasma.

Anthrax.—The bacillus in some cases may be isolated from the blood. No marked blood changes have been noted.

Yellow fever.—In this disease the anæmia is not so much a result of erythrocyte as hæmoglobin loss. Normoblasts and other qualitative changes occur in the erythrocytes. There may be hæmoglobinæmia. The blood coagulates slowly and fibrin is diminished. This is one of the few infectious diseases in which there is no leucocytosis, but rather leucopœnia. According to Gray[2] there is no increase of mononuclear cells, but rather a decrease, and this is a point of diagnostic importance between yellow and malarial fever.

Malta fever.—Secondary anæmia occurs, especially if there be hæmorrhage. Erythrocytes are diminished. The leucocytes are said to be normal. Serum reaction with *bacillus melitensis* is positive and the opsonic index low.

Epidemic cerebro-spinal meningitis.—The meningococcus has been isolated from the blood in some cases. The opsonic index of the blood in relation to the organism is usually high, and according to Houston and Rankine[3] said to be of distinct diagnostic value. Alice Taylor[4] confirms this, and considers that the high index is pathognomonic of the disease. The erythrocytes are not necessarily altered, but a high count is sometimes observed, due to vasomotor influences. Leucocytosis is usual, and the more severe the disease the higher the count.

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[1] *Deut. Med. Woch.*, 1897, vol. xxiii, p. 560.

[2] *Brit. Med. Journ.*, January 25, 1902.

[3] *Lancet*, May 4, 1907.

[4] *Lancet*, July 6, 1907.

LOCAL AND GENERAL SEPTIC INFECTIONS

The blood changes in processes included under the above headings may be due to absorption of toxins from local lesions, or the presence of organisms in the blood stream, with their associated toxins.

The presence of organisms, e.g. pyogenic cocci, in the blood is a matter of extreme gravity. An exception to this is the finding of staphylococcus albus, which is of less importance. Pyogenic organisms may be isolated from the blood in many septic conditions, and in cases suggestive of septic infection the blood should always be examined for them.

The condition of the blood in septic infections of different kinds is practically alike, so that a general account will suffice. Marked anæmia is the rule, especially in severe cases; its advent is rapid and it lasts long.

The fall is in both erythrocytes and hæmoglobin, with a low colour index. Qualitative changes in the erythrocytes occur and erythroblasts may be found. Hæmoglobinæmia takes place and produces staining of the tissues. Leucocytosis is nearly always present, especially in cases of moderate severity, while in very mild or virulent infections it may not occur. I have noted in a case of streptococcal infective endocarditis, in which the organism could be repeatedly cultivated from the blood, leucocytosis was never present. The rise is due to neutrophiles, lymphocytes being diminished and eosinophiles even disappearing altogether. Myelocytes and stimulation forms may appear. Iodophilia is usually marked. In ordinary cases the fibrin is increased, in severe diminished. Leucocytosis always occurs in active pus formation, but where purulent collections are walled off so as to effectually prevent absorption it may be slight or wanting. In some cases, e.g. gonorrhœal, there may be a rise in eosinophiles.

CONSTITUTIONAL DISEASES AND DUCTLESS GLANDS

Diabetes.—The alkalinity of the blood is reduced, especially in diabetic coma. Sugar is in excess, and the blood sometimes contains excess of fat, which may give it a creamy appearance. The blood when spread in films and fixed at a temperature of 135° C. and allowed to cool, exhibits a lessened affinity for stain. In this test, introduced by Bremer, thick films are used and stained in a 1 per cent. aqueous solution of Congo red, freshly made; washed and dried between filter paper. Diabetic blood remains yellow, normal is stained red. Methylene blue may be used; the diabetic blood becomes yellowish-green, normal blue. It is not regarded as absolutely reliable. The same yellowish-green colour I have noted in pernicious anæmia.

Williamson's test is carried out by mixing the blood with an alkaline solution of methylene blue—

Blood	20 c.mm.
Aq. sol. meth. blue, 1 : 6,000 . .	1 c.c.
Liq. potassæ (60 per cent. sp. gr. 1058) .	40 c.c.
Aq. dest.	40 c.c.

Mix and stand for three to four minutes in boiling water in a beaker. Diabetic blood turns the solution yellow, normal blood does not.

The amount of anæmia varies, and is supposed to be dependent upon the degree of diuresis, so that the erythrocytes may be found normal, increased or decreased in number. A high digestion leucocytosis occurs in some cases. Myelocytes may be present. Glycogen is present in many leucocytes, and extracellular iodophilia is common.

Gout.—During the acute stage fibrin is increased. There are no special blood changes, there may be anæmia in the poor and ill-fed. Leucocytosis occurs, occasionally eosinophiles are relatively increased, and myelocytes may appear. The examination for uric acid in the blood may be carried out by adding to serum, 6 drops of a 28 per cent. aqueous solution of acetic acid to each drachm, and placing in it a fine thread; after allowing it to stand for eighteen to twenty-four hours crystals of uric acid are found adhering to the thread. The result is not always positive, nor is it special to gout.

Myxœdema.—There are no definite blood changes.

Anæmia is generally due to loss of hæmoglobin, but in some cases the colour index is raised. Large erythrocytes may be seen, and occasionally myelocytes appear.

Exophthalmic goitre.—The blood may be normal, or a certain degree of chlorosis may be present. Lymphocytes and eosinophiles are occasionally relatively increased. In cases examined for me by Dr. Pentland Hick in the Royal Infirmary, Liverpool, the calcium index as determined by Dr. Blair-Bell's method has been found as low as 0·5 of the normal.

Addison's Disease.—There is nothing characteristic about the blood changes. In some cases there may be severe anæmia with erythrocyte degeneration. Leucocytes may be normal or reduced; occasionally the lymphocytes and eosinophiles are relatively increased, and a few myelocytes and basophile cells found.

Osteomalacia.—The alkalinity of the blood which was formerly regarded as lessened is now said to be unaltered. A moderate degree of anæmia of chlorotic type is usual. Lymphocytes and eosinophiles are sometimes increased, and a few myelocytes are said to occur occasionally.

Rickets.—Anæmia is always present, with a disproportionate fall in hæmoglobin and a low colour index. Leucocytosis is always more marked in children than in adolescents, and is usually present. It is difficult to gauge the true amount of leucocytosis in children, as so much depends upon their vigour and development. As has been pointed out before, the blood of children is easily altered. Qualitative changes may occur in the erythrocytes, and normoblasts appear. Lymphocytosis is common, myelocytes may be present, and the eosinophiles increased.

MALIGNANT DISEASE

The blood changes in malignant disease are variable. In the early stages there may be little or no alteration, and this particularly in small and slowly growing tumours. Sooner or later anæmia supervenes, and this occurs early in rapidly growing and extending tumours especially associated with metastases. The most constant and characteristic change is the loss of hæmoglobin. The erythrocytes rapidly diminish in advanced cases. As a rule the colour index is low. Qualitative changes occur in the erythrocytes, they are small in size, degenerated and deformed with polychromasia and basophile granulation. Normoblasts are not uncommon and megaloblasts may appear. The erythrocytes are said to be more vulnerable than usual. After operation the hæmoglobin first falls and then rises, but is said not to reach its previous level.

Cabot classifies cases of malignant disease in three groups : (1) those with normal blood ; (2) those with normal erythrocytes but loss of hæmoglobin ; (3) those with loss of both erythrocytes and hæmoglobin. Leucocytosis is the general rule. According to Cunliffe [1] the leucocytosis is greatest in cachectic cases and those with metastases, and he considers a relative increase of neutrophiles even without leucocytosis to be suggestive of cancer. Myelocytes occur in small numbers in advanced cases. Marcotte [2] considers an increase of mononuclears a constant feature, and that a proportionate increase of neutrophiles is not to be held as of importance in the diagnosis of cancer.

The leucocytosis is said to fall after operation and reappear with recurrence of the growth. The onset and degree of leucocytosis seems to be influenced by the presence of inflammatory changes in the tumour or its vicinity. Coagulation and fibrin formation remain normal, unless there be ulcerative or inflammatory processes to increase it. The specific gravity follows the hæmoglobin loss. Sugar has been found in excess in the blood in some cases of visceral carcinoma. The blood changes associated with sarcoma are similar to those of carcinoma, but are generally regarded as more pronounced.

[1] *Med. Chronicle,* September, 1903.

[2] *Thèse de Paris,* 1902

TUBERCULOSIS

The serum agglutinating reaction in tuberculosis is positive in many cases, and although it has been regarded as of doubtful clinical value, Arloing and Courmont[1] with others, regard the agglutination as specific, and advise that in those cases in which it is positive and no other clinical evidence available so that the reaction may have been regarded as erroneous, a more thorough examination may reveal the presence of tuberculosis. Positive reactions are said to occur in other diseases. It is surprising how slight the blood changes usually are in this disease. Erythrocytes and hæmoglobin often remain normal, in some cases there is hæmoglobin loss, in others loss of both. Qualitative changes in the erythrocytes are rare, and even after hæmoptysis normoblasts seldom appear. Pure tuberculous infection has little or no effect upon the blood, and if changes do occur they are generally the result of infections with pyogenic organisms, when qualitative changes appear according to the degree of anæmia. The onset of such secondary infections is largely a matter of anatomical site and ease of access of other organisms. From their investigations into a large series of cases of pulmonary tuberculosis before and during Sanatorium treatment Bardswell and Chapman[2] conclude that (1) In cases of early and limited pulmonary tuberculosis with little or no fever the erythrocyte count and hæmoglobin content are within normal limits, or there is a slight hæmoglobin loss. In some cases the erythrocyte count may be from 6,000,000 to 7,000,000 per c.mm. (2) That patients with extensive and active lesions and much fever, but no other complications show nearly always a numercial increase in erythrocytes to 6,000,000 per c.mm.; at the same time there is hæmoglobin loss with a colour index about 0·75. (3) The greater the activity of the lung disease, and the longer its duration, the more marked is the hæmoglobin reduction and the increase of erythrocyte count. (4) Gradual arrest of the tubercular process is associated with a rise of hæmoglobin and fall of erythrocytes. (5) Complete arrest of the lung disease is associated with a return of the blood to normal. (6) The blood of patients with completely arrested disease and of normal people when living in Sanatoria becomes very rich in hæmoglobin, the hæmoglobin content in both cases being usually considerably higher than that of normal people living in towns. The same applies to the leucocyte count; as a general rule in pure tuberculosis there is little or no leucocytosis, the lymphocytes are often relatively increased, especially in children; the onset of leucocytosis denotes a secondary infection of some sort.

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[1] *Boston Med. and Surg. Journ.*, December, 1904.

[2] *Medico-Chirurgical Transactions*, vol. xc, 1907.

This general rule is applicable to all anatomical varieties, but in some it is altered more easily than others, for instance the lungs and genito-urinary system. Even in tuberculous glands there may be no leucocytosis unless access to secondary septic infections occur through sinuses, or if an abscess form and is opened or should burst. In acute miliary tuberculosis a leucocyte rise indicates a secondary infection. The principal exception to this rule is seen in tuberculous meningitis in which leucocytosis is present in the majority of cases, but it has been suggested by Cabot that if in a case of meningitis of unknown origin leucocytosis is absent, it will probably prove to be tuberculous.

SYPHILIS

The *spirochœta pallida* is present in the blood, although the conditions for its existence there are not good, and its demonstration exceedingly difficult. Its presence has been proved by inoculation. Anæmia is a constant feature in syphilis. Both erythrocytes and hæmoglobin are markedly diminished, especially after the advent of secondary symptoms. The hæmoglobin sinks rapidly from the date of the primary inoculation, but the erythrocytes are not proportionately altered until the eruption takes place. The blood change is more marked in women than men. The degree of anæmia seems to depend upon the intensity of the infection. In some tertiary conditions and in congenital cases a high grade anæmia may be present resembling morphologically pernicious anæmia. Mercurial treatment has a beneficial effect upon the blood for a time, but anæmia returns if it be persisted in, and in those cases in which the blood deterioration goes on in spite of treatment, severe tertiary lesions are almost sure to follow. Justus claims that, if mercury be administered either by inunction or injection before the secondary stage, the hæmoglobin first falls and within twenty-four hours then rises again to a higher point in the course of a few days. This, he holds, does not obtain in other diseases.

In a private communication made to me by Dr. Mackenna, of Liverpool, and not yet published, he informs me that in a number of cases which he had examined during the secondary stage before mercurial treatment had been instituted the erythrocytes were much reduced. On the administration of iron they rose gradually, but after the injection of a single dose of grey oil there was a sudden large increase within three or four days, coincident with a marked diminution in the number of spirochætæ discoverable in the local lesions. Dr. Mackenna also believes that the spirochætæ have a direct destructive influence on erythrocytes. Other observers have failed to confirm Justus' reaction. It may be regarded as positive in many cases, but it is limited to the primary stage of the disease.

Leucocytes are normal or only slightly increased in the primary stage; during the eruptive stage the lymphocytes are increased and there is a moderate leucocytosis. Mast cells may be numerous and myelocytes may appear occasionally. In the tertiary stage leucocytosis may be more marked, with increase of lymphocytes; myelocytes may appear. Eosinophilia is said not to occur in syphilis, but the reports are conflicting. Tomomitsu Watabiki[1] finds that the total count of leucocytes in the tertiary stage is usually decreased, and that eosinophiles are present in greater numbers than in other stages. The condition of the blood, as a whole, may be taken as an index of the severity of the disease.

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[1] *Amer. Med.*, April, 1907.

LEPROSY

In early cases of leprosy there is little or no blood change. In severe cases anæmia may be profound, resembling pernicious anæmia with a high colour index and the presence of megalocytes. There may be an increased percentage of lymphocytes and eosinophiles. The specific bacilli have been found in the blood, and a positive serum reaction obtained.

DISEASES OF THE LUNGS AND PLEURÆ

Bronchitis.—There is generally some degree of secondary anæmia in acute cases and moderate leucocytosis may be present. In chronic bronchitis there are no special changes. In capillary bronchitis the blood changes approach those of acute pneumonia. In septic suppurative conditions, such as occur in bronchiectasis, leucocytosis may be expected.

Emphysema and asthma.—A fair degree of polycythæmia in the peripheral circulation may arise in cases associated with cyanosis. I have found the erythrocyte count up to 6,800,000 and it may reach more than this. The viscosity is increased. Eosinophile cells are numerous in the blood, as in the sputum, in asthma, especially during the paroxysms. Occasionally eosinophilia may be constant.

Serous pleurisy.—Except in chronic cases there may be no change in erythrocytes or hæmoglobin. Leucocytosis may occur in the early stage, but falls again, so that in a case of well-marked effusion it may be absent. It is a fluctuating quantity. Should leucocytosis persist it indicates some complication. The low leucocyte count commonly found may be due to the fact that a large percentage of serous pleuritic effusions are tuberculous.

Purulent pleurisy (empyema).—There is an associated anæmia of secondary type. Leucocytosis is constant and iodophilia present. If the pus be withdrawn the leucocyte count falls to rise again as the pus re-accumulates.

Acute pneumonia.—*Vide* acute infectious diseases.

Pulmonary tuberculosis.—Much of what has been noted under tuberculosis is applicable to this disease. In incipient cases leucocytosis is absent except after hæmoptysis. Secondary infections and cavitation are associated with leucocytosis. Fibroid cases, unless with cavitation, do not show leucocytosis. In ordinary cases the lymphocytes are in the majority, but when definite leucocytosis occurs it is neutrophile in type. Injections of tuberculin are said to produce eosinophilia, so may auto-inoculation from local lesions. In advanced cases myelocytes may be found. Dr. Forsyth at the Liverpool Consumption Hospital has found tubercle bacilli in the blood in a large proportion of cases.

DISEASES OF THE CIRCULATORY SYSTEM

Acute endocarditis.—In many cases no blood changes occur other than those associated with acute rheumatism.

Ulcerative or malignant endocarditis.—Infective organisms may be isolated and cultivated from the blood in a large number of cases. The blood change is similar to those described under general septic infections. Marked anæmia is a constant feature with rapid erythrocyte and hæmoglobin loss. Qualitative changes are present in the erythrocytes, and hæmoglobinæmia may occur. Leucocytosis is the general rule, but of slight degree, and in some cases of streptococcal origin it may be absent. In one streptococcal case cultures were made with certainty at each bacteriological examination, but I found a more or less persistent leucopœnia. The leucocyte count fluctuates very much in this disease.

Chronic valvular disease.—In well compensated cases there is no blood change, but with failure of compensation there may at first be "serous plethora," due to lowered blood pressure and diminished excretion of fluids, so that the specific gravity falls and the peripheral blood shows a fall in hæmoglobin and erythrocytes. When stasis is established the peripheral blood may show polycythæmia with increased specific gravity and viscosity. In aortic disease there is usually a certain degree of anæmia. According to Cabot, mitral disease is often associated with anæmia and leucocytosis. As a rule, there is no leucocytosis, unless complications are present. Congenital heart lesions are generally associated with marked polycythæmia and some leucocytosis.

Pericarditis.—The blood changes may be those of acute rheumatism in which there is a leucocytosis, and this occurs in septic effusions in contrast to tubercular pericarditis in which leucocytosis is absent. In a case of extensive hæmorrhagic effusion of septic origin I found a leucocytosis of 60,000 per c.mm.

DISEASES OF THE DIGESTIVE SYSTEM

Mouth.—Acute septic conditions may produce a leucocytosis, and oral sepsis is very commonly associated with secondary anæmia, often of high grade, and also of the pernicious type.

Malignant disease of the œsophagus.—Secondary anæmia may arise, and leucocytosis in some cases, though not common, unless associated with complications. From restricted inhibition of fluid a certain amount of polycythæmia may be present though transient.

Acute gastritis.—There are no special changes in erythrocytes or hæmoglobin. If there be much vomiting a transient polycythæmia may occur. There is often leucocytosis which comes on rapidly.

Chronic gastritis.—Anæmia is often present and may be extreme. Chronic gastritis with atrophy of the glands has been given a place in the etiology of pernicious anæmia. Leucocytosis is absent and there may be leucopœnia in some cases. Digestive leucocytosis is retarded or may be absent in a certain proportion of cases.

Dilated stomach.—Anæmia may be present, but where there is vomiting of large quantities of fluid, often in excess of ingestion, the blood may show some degree of concentration. Digestive leucocytosis may be absent.

Gastric ulcer.—Severe anæmia is common, with erythrocyte and hæmoglobin loss and a low colour index. Cabot is of the opinion that in no disease except pernicious anæmia may the erythrocytes fall so low, not even in cancer of the stomach. This especially applies to cases associated with hæmatemesis, not to those without it. Excessive vomiting may keep the erythrocyte count up. Qualitative changes in the erythrocytes may be marked and normoblasts occur. Leucocytosis is absent, unless there be hæmorrhage; transient after digestion, its presence otherwise indicates some complication such as perforation. After rectal feeding for some time the first ingestion of food into the stomach may produce a marked digestion leucocytosis.

The blood changes in duodenal ulcer are practically the same.

Cancer of the stomach.—Marked anæmia is the rule, with a low erythrocyte count. In a small number of cases associated with much vomiting the count may be high, but in the majority of cases it falls to about half the normal but rarely below 1,500,000 per c.mm. Qualitative changes in the erythrocytes are present, but megaloblasts are rarely if ever seen. The colour index is low. Leucocytosis is not frequent unless in advanced cases with metastases, when it is nearly always present. Digestion leucocytosis is often absent, but is too inconstant a sign to be of diagnostic value, and differential leucocyte counts do not help.

Acute enteritis.—If there be excessive diarrhœa with watery stools the erythrocyte count and specific gravity may be raised. Slight leucocytosis is generally present, which in infants is usually lymphocytic in character.

Chronic diarrhœa.—Here a gradual drain of albuminoids is exercised upon the blood and marked anæmia follows, both erythrocytes and hæmoglobin being diminished and the colour index low. Qualitative changes take place and normoblasts, occasionally megaloblasts, may appear. Leucocytosis is rare. Hæmorrhage from the bowel intensifies the anæmia.

Intestinal obstruction.—The chief interest lies in the fact that leucocytosis is rapid in its onset. A high count is said to denote gangrene ; when gangrene or peritonitis is established a leucocyte fall takes place. There is generally a fall on the fifth day.

Appendicitis.—The erythrocytes show no change except in chronic cases with long-standing abscess. Coagulation is often slow, but is said to be increased in suppurative cases. In very mild or severe cases leucocytosis may be absent, also when associated with general suppurative peritonitis. An increasing leucocytosis indicates a spreading process and if it goes on is a bad sign, and more important than a high count which does not increase. An abscess thoroughly encapsulated may be unaccompanied by leucocytosis. In ordinary cases the leucocytosis declines with resolution of the local process. Cazin and Gros [1] state that leucocytosis is usually present from the beginning of the appendicitis whether there be suppuration or not, and the character of the lesion cannot be judged from an ordinary enumeration of the leucocytes. It must be repeated often, and when the leucocytes are permanently and considerably increased suppuration is present. The leucocyte count is more important than the temperature relative to operation. They consider that in generalized peritonitis leucocytosis is a favourable sign, and that in peritonitis with hypertoxæmia leucocytosis does not occur. The persistence of leucocytosis after operation indicates undrained foci of suppuration. Qualitative changes may be present in the leucocytes, and I have noted marked alterations in staining affinity, lessened granulation in the neutrophiles, and the presence of many transitional forms.

Peritonitis.—This may be conveniently considered here. Much of what has been said of appendicitis is applicable to it. Any degree of anæmia which may arise depends largely upon the form of peritonitis and its chronicity. In a few cases associated with vomiting the erythrocyte count may be raised. In severe cases both erythrocytes and hæmoglobin may fall rapidly. Fibrin is increased. Leucocytosis is present except in hypertoxic and fulminating cases. As the peritonitis extends the leucocytosis increases. In tuberculous cases there is usually no leucocytosis. Carcinoma is usually associated with leucocytosis.

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[1] *Med. Press,* September 30, 1903.

Pancreatitis.—In chronic cases there is a definite anæmia of secondary type without leucocytosis. In some cases there may be leucopœnia. Hæmorrhagic lesions are generally accompanied by leucocytosis.

DISEASES OF THE LIVER

Jaundice.—In jaundice the serum is coloured with bile pigments. In the simple catarrhal form the erythrocytes may not be numerically altered; a slight leucocytosis may occur. In chronic obstructive jaundice there is a fall in erythrocytes and hæmoglobin with leucocytosis. In both, coagulation is slow. Without alteration of fibrin rouleaux formation is feeble and crenation takes place quickly. Endoglobular degenerative changes occur, and the individual erythrocytes are large in size. The specific gravity is raised and the alkalinity diminished. The reduction in coagulation power is of importance in reference to surgical operations, as profuse bleeding occurs which adds to the gravity of the procedure.

Acute yellow atrophy.—There is a slight degree of polycythæmia with leucocytosis and a low colour index. This is similar to what occurs in poisoning by phosphorus, but in less degree.

Gall stones.—Coagulation is said to be retarded. There is no change in erythrocyte count or hæmoglobin and no leucocytosis unless there be inflammation.

Cholecystitis and cholangitis.—Coagulation is increased; so the fibrin. Leucocytosis is well marked. It passes off as the inflammation subsides.

Abscess.—In chronic abscess leucocytosis may be absent, in acute it is marked as a rule. With chronic abscess secondary anæmia develops. Rogers[1] emphasizes the value of blood counts in differentiating acute hepatitis from amœbic abscess. He compares the ratio of leucocytes to erythrocytes in the two conditions. In acute hepatitis the average of four cases gave a ratio of 1 to 650; in abscess, the average of ten cases being 1–260. He concludes that absolute leucocytosis is nearly always present in amœbic abscess, but in chronic cases with marked anæmia the leucocytosis is usually relative. The degree of leucocytosis is greatest in acute cases, slight in insidious cases. In acute hepatitis without suppuration, leucocytosis is nearly always absent.

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[1] *Brit. Med. Journ.*, November 11, 1905.

Cancer.—There is marked anæmia with lowered erythrocyte count and leucocytosis in a large proportion of cases.

Cirrhosis.—There is a numerical decline in both erythrocytes and hæmoglobin, especially in the hypertrophic form. The colour index in atrophic cirrhosis is low, but in hypertrophic cirrhosis with jaundice it is often above 1. Degenerative changes take place in the erythrocytes. In Hanot's cirrhosis megalocytosis may occur. Leucocytosis is not common in the atrophic form unless toxæmia be present, whereas it is usually met with in the hypertrophic variety. It is neutrophile in character.

Hydatid disease.—Eosinophilia is the rule especially in cases unassociated with suppuration, in the latter the eosinophile leucocytosis usually gives place to a neutrophile increase.

DISEASES OF THE GENITO-URINARY SYSTEM

In the nephritides several factors enter into the production of such blood changes as occur, chief of which are hæmorrhage, albuminuria, cardio-vascular disturbances and, as in scarlet fever for example, the specific nature of the disease which is associated with the nephritis.

Acute nephritis.—Anæmia comes on rapidly in a large proportion of cases with a marked fall in erythrocytes and a still greater depression of the hæmoglobin reading. The colour index is low. Fibrin and blood platelets are increased and the specific gravity lowered. Leucocytosis is present with uræmia; it may persist in hæmorrhagic or uræmic cases. In scarlatinal nephritis it may last for a long time, a peculiarity of this exanthem. Eosinophiles are said to disappear.

Chronic parenchymatous nephritis. — Erythrocytes are numerically diminished in a large proportion of cases, in some slightly, in others markedly. The hæmoglobin is lowered to a greater degree than in the acute disease. The colour index is low. Polycythæmia may occur in certain cases. Fibrin is increased. The specific gravity is lowered, and the alkalinity lessened, especially before the onset of uræmia. Leucocytosis is present with uræmia, but not otherwise.

Chronic interstitial nephritis.—There is no special change in the blood before the development and establishment of cardio-vascular changes, when they are the same as those associated with chronic valvular disease and failing compensation. The leucocytes are unaltered.

Pyelonephritis and pyonephrosis.—As a rule, these conditions are accompanied by severe anæmia with erythrocyte and hæmoglobin loss. The colour index is low. Leucocytosis is the rule.

Urethritis.—Gonorrhœal urethritis shows no special blood change with the exception of a certain degree of leucocytosis. The eosinophile cells are said to be increased in a large number of cases.

Tuberculosis.—Tuberculosis of the genito-urinary organs is accompanied by the usual blood changes of tuberculosis, but secondary infections are so common that the usual condition common to tubercle is masked by that produced by pyogenic organisms, so that leucocytosis is present in a large proportion of cases.

INTESTINAL PARASITES

Bothriocephalus latus.—Anæmia is practically always present and may be very severe. There is a marked loss of erythrocytes and hæmoglobin with a colour index above 1 in the majority of cases. Qualitative changes in the erythrocytes are present in the form of megalocytosis, poikilocytosis, polychromasia and basophile stippling. Megaloblasts and normoblasts are present, the former predominating. Blood platelets are diminished. Leucocytes are normal or diminished, a few myelocytes may be found; eosinophilia is rare. Thus the blood is identical with pernicious anæmia, but returns to normal on expulsion of the worm.

Ankylostomiasis.—The effects of this worm upon the host is to produce marked anæmia, in some cases very severe. "Egyptian chlorosis," miners' anæmia, brickmakers' anæmia, tunnel anæmia and mountain anæmia are due to the ankylostomum duodenale.

The hæmoglobin and erythrocyte readings are often very low in severe cases, the former lower than the latter, so that the anæmia is chlorotic in type with a low colour index. According to Haldane and Boycott,[1] the total volume of the blood and the hæmoglobin are both greater than normal, with a low colour index, thus differing from pernicious anæmia in which the volume and hæmoglobin are reduced and the colour index high. Qualitative changes take place in the erythrocytes with poikilocytosis and altered staining reaction. Ashford [2] found that megaloblasts never predominate over normoblasts. Morphologically the erythrocyte changes resemble pernicious anæmia, but is never quite as marked. The leucocytes may be normal or subnormal, but the characteristic feature is the presence of eosinophilia to a greater or lesser degree. This is most marked in early cases, lowest in long-standing cases [3] and those which end fatally.[4] The presence of eosinophilia is considered a favourable prognostic sign. The eosinophilia is both absolute and relative. A neutrophile leucocytosis from any cause generally determines a fall in eosinophiles.[5]

Boycott,[6] in a more recent paper on "Anæmia in Ankylostomiasis," points out that only a comparatively small proportion of those infected with ankylostoma suffer from anæmia, although eosinophilia is common. The symptoms associated with anæmia are never so severe in ankylostomiasis as in pernicious anæmia; and that when removed from the source of infection the anæmia tends to natural cure, which is not so in pernicious progressive anæmia. In pernicious anæmia the colour index is above 1, in ankylostomiasis it is low and depends upon the hæmoglobin fall. Eosinophilia is diagnostic of ankylostomiasis, not of pernicious anæmia, and qualitative changes are more pronounced in the latter. He found the blood volume twice the normal in three cases, and the condition he considers one of hydræmic plethora. The anæmia is more apparent than real, due to the dilution of the blood, the total hæmoglobin content being practically normal and the total number of red cells in excess of normal.

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[1] *Journ. of Hygiene,* 1903, vol. iii, no. 1.

[2] *Amer Med.,* 1903, p. 391.

[3] Boycott and Haldane, *Journ. Hygiene,* 1903, vol. iii, no. 1.

[4] *Amer. Med.,* 1903, p. 391.

[5] Boycott, *Journ. Hygiene,* 1904, p. 439.

[6] *Brit. Med. Journ.,* November 9, 1907.

Trichiniasis.—There is no anæmia as a rule, but it may occur in protracted cases; there is rather a tendency to high erythrocyte counts and hæmoglobin readings. Since the discovery by T. R. Brown[1] of the occurrence of eosinophilia in trichiniasis, the phenomenon has been repeatedly corroborated, and this blood change is a most marked feature in the disease. The gain is absolute and relative, and at the expense of the other leucocytes. It generally passes off as the disease becomes quiescent. In some few cases it may not occur.

Other forms of helminthiasis.—In addition to those mentioned above, blood changes may arise in connexion with other forms of helminthiasis. Anæmia may occur in cases infected with trichocephalus dispar, ascaris lumbricoides. Eosinophilia may also occur in other forms of tæniæ, and in ascariasis.

DISEASES OF THE NERVOUS SYSTEM

Neuritis.—Neuritis from whatever cause may be associated with anæmia in which both erythrocytes and hæmoglobin are decreased. There is usually a degree of leucocytosis, but this does not occur regularly in post-diphtheritic neuritis and not at all in plumbism.

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[1] *Johns Hopkins Hosp. Bull.*, 1897, vol. iii. p. 79.

In herpes zoster leucocytosis occurs in many cases. In simple neuralgias there is no blood change.

Meningitis.—There need not be any alteration of erythrocytes, but the hæmoglobin may be lowered. Neutrophile leucocytosis is the rule in purulent cases of septic origin, but it is inconstant in tuberculous meningitis. Sometimes a relative increase of lymphocytes occurs and eosinophiles may be decreased. (*Vide* tuberculosis, cerebro-spinal meningitis.)

Diseases of the brain.—Leucocytosis occurs in abscess and cerebral hæmorrhage, but in tumour it is usually absent or slight.

Diseases of the cord.—The association of combined sclerosis with profound anæmia, e.g. pernicious, is established. In chronic cord diseases there are no special changes, but in acute myelitis leucocytosis is usually present.

Mental diseases.—Mental diseases are in many cases associated with anæmia, but not necessarily the result of it.

In **general paralysis of the insane** anæmia develops. It is moderate and progressive, the hæmoglobin especially falls. In the terminal stages Diefendorf [1] finds a rise in erythrocytes, hæmoglobin and neutrophiles. The condition is generally associated with leucocytosis during convulsive and apoplectic attacks. The relative proportions of the leucocytes is inconstant, but a relative increase of large mononuclear and occasionally of eosinophile cells is common. Capps [2] describes a mononuclear neutrophile cell resembling a small myelocyte as occurring in this disease. Qualitative changes in erythrocytes may occur. During convulsive stages both erythrocytes and hæmoglobin may be raised.

Ford Robertson and McCrae [3] attribute this disease to infection by a bacillus which they term the *bacillus para-*

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[1] *Amer. Journ. Med. Sciences*, December, 1903.

[2] *Amer. Journ. Med. Sciences*, 1896, vol. cxi, p. 650.

[3] *Rev. Neur. and Psych.*, February, March, April, 1906.

lyticans, and which they have been able to isolate from the blood, they recognize solvent, lysogenic or bacteriolytic action of the serum on this organism. The opsonic power of the blood to *bacillus paralyticans* varies considerably.

Acute delirium.—Leucocytosis occurs. In the delirium of puerperal septicæmia there is leucocytosis which is not present in puerperal mania.

Acute mania.—Lewis Bruce[1] is of the opinion that acute continuous mania is due to toxæmia. There is a high grade leucocytosis, the higher it is and the greater the percentage of neutrophiles the more favourable the outlook, an increase indicating recovery. It persists after recovery. Cases which do not recover rapidly show a fall in the original leucocytosis to a lower level at which it remains.

Dementia.—Anæmia is generally present, leucocytes normal or slightly raised.[2]

Chorea.—The most constant change is an increase of eosinophile cells.

Epilepsy.—A moderate degree of anæmia may be present of chlorotic type. Leucocytosis occurs during convulsive attacks. According to Dide, the neutrophiles fall and the eosinophiles rise immediately before the fit; the neutrophiles rise immediately after it, producing a temporary leucocytosis.

Functional diseases.—There are no characteristic changes other than loss of hæmoglobin. In hysteria, polycythæmia an increase in eosinophiles sometimes occurs. Relative lymphocytosis has been noted in neurasthenia. The value of blood examination in functional disease is in the negative evidence it affords.

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[1] *Journ. Mental Sciences,* April, 1903.

[2] For a detailed account of the blood conditions found in the insane the reader is referred to the discussion on the subject held at the Lille Congress, 1906, opened by Dide. *L'Informateur des Alienistes et des Neurologists,* August, 1906. *Gaz. d. Hôp.,* August. 9. 1908. *Progrès Méd.,* August 11, 1906.

THE HÆMORRHAGIC GROUP OF BLOOD DISEASES

The blood changes in the hæmorrhagic group of blood diseases are not characteristic or constant. They resemble to a large extent the ordinary changes associated with hæmorrhage from any cause. How far the blood change alone is responsible for the hæmorrhages which occur is not decided, and in some measure the blood vessels are at fault. Lack of fibrin formation is not by any means constant, and coagulation may remain unaltered. The tendency to hæmorrhage is probably due to alteration in the blood and vessel-wall. Of chemical agents causing hæmorrhage, bacterial products take a prominent place, but many poisons, phytotoxins (plant poisons), zootoxins (animal poisons), e.g. snake venom, are specially effective. These may act upon blood or vessel-wall or both.

Hæmophilia.—The blood changes are not characteristic of the disease, but are common to all secondary anæmias. The white cells are decreased with a relative increase of lymphocytes. Blood platelets are decreased. No constant alteration in the salts, water, alkalinity, or fibrin formation have been found. Between the attacks the rate of coagulation is slower, during the bleeding it may reach or be more rapid than normal. Sahli suggests that the persistency of the bleeding may be due to lack of local fibrin forming substances in the cells of the vessel-walls. Emile-Weil[1] recognizes two varieties of the disease, sporadic and hereditary. In the former the blood is very fluid, coagulation time lengthened, clot and serum abundant; in the latter the blood is viscous; coagulation time much lengthened, the clot soft, serum less abundant, and there is leucopœnia.

Purpura hæmorrhagica.—The blood condition is practically that of post-hæmorrhagic anæmia, with loss of erythrocytes and hæmoglobin, qualitative changes in the erythrocytes, and leucocytosis. In some cases both cell changes may be extreme. The specific gravity is diminished. Methæmoglobin may be present. Blood platelets are diminished or absent. Bensaude[1] states that in severe cases there is no separation of clot and serum, but it takes place in mild cases. Hayem has also noticed this peculiarity with diminished fibrin formation but the coagulation rate normal.

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[1] *Gaz. d. Hôp.*, November 5, 1906.

Scurvy.—There is nothing characteristic further than the blood changes of secondary anæmia. The hæmoglobin suffers loss more than the erythrocytes. Leucocytosis is not usually present, unless from complications or hæmorrhage. In infantile scurvy the anæmia may be extreme.

Paroxysmal hæmoglobinuria.—The blood change is that of anæmia with loss of erythrocytes and hæmoglobin. Blood platelets are said to be increased. Coagulation is rapid but transient, and solution of the clot soon follows. The erythrocytes may be found broken up. Hæmolysis takes place, with hæmoglobinæmia. Eason[2] has shown that there is a toxic substance in the serum which is hæmolytic to the patient's own erythrocytes, and also to those from a healthy subject, and that it acts upon the erythrocytes best at a low temperature, the hæmolysis taking place when the temperature is subsequently raised.[3] This accounts for attacks following exposure to cold.

Blood changes following severe burns.—The erythrocytes become fragmented and distorted, a loss of water takes place, and an increase of 1,000,000 or more per c.mm. in the number of erythrocytes may take place rapidly. In fatal cases the polycythæmia may be very marked, even up to 4,000,000 per c.mm. There is also an increase of blood platelets and a high grade leucocytosis. The neutrophile increase is not so pronounced as in inflammatory leucocytosis.[4] Hæmoglobinæmia is present. The blood change is said to be due to toxic materials formed in the burnt area and absorbed into the blood. They do not take place if the burnt area is removed or absorption is prevented by local thrombosis in the vessels.

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[1] *La Semaine Med.*, 1897, p. 21.

[2] *Edin. Med. Journ.*, 1906, vol. xix, p. 43.

[3] *Journ. Path. and Bact.*, vol. xi, No. 2, 1906.

[4] Locke, *Boston Med. and Surg. Journ.*, 1902.

DISEASES OF THE SKIN

The chief blood change noted, and one to which much importance has been assigned, has been the presence of eosinophilia, especially in skin diseases with herpetiform and bullous eruptions. The constancy and diagnostic value of this change has been questioned by recent observers. H. S. French,[1] from an investigation into the frequency of eosinophilia in skin diseases, concludes that in cases other than dermatitis herpetiformis and pemphigus it is either slight or absent. Lams,[2] after examining the literature bearing upon these two and allied bullous diseases, concludes that eosinophilia is of no diagnostic value in differentiating them.

BLOOD CHANGES DUE TO THE ACTION OF CERTAIN POISONS

Many poisons act indirectly upon the blood by their effects upon various tissues and organs, but others have a specific action upon the blood itself, and produce rapid and definite changes. They are conveniently divided into four main groups.

Group I

Those which combine with the hæmoglobin and destroy its function as an oxygen carrier, but without hæmolytic action upon the erythrocytes. In this group the principal examples are carbon-monoxide (illuminating gas), sulphuretted hydrogen and hydrocyanic acid.

Carbon-monoxide combines with the hæmoglobin to form carboxyhæmoglobin, which is a very stable compound, imparting to the blood a characteristic bright cherry-red colour, and preventing coagulation, so that it remains fluid. The blood yields a spectrum with two absorption bands resembling oxyhæmoglobin, but slightly nearer to the violet end of the spectrum (Fig. 8). The spectrum does not alter on the addition of the usual reducing agents if the blood be saturated with the gas. Polycythæmia is usually present, and a certain degree of leucocytosis ; normoblasts and myelocytes may be present. In chronic cases anæmia occurs.

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[1] *Brit. Med. Journ.*, April 20, 1902.

[2] *Rev. de. Méd.*, June 10, 1907.

Sulphuretted hydrogen.—The blood is fluid and dark in colour. Sulphur-methæmoglobin is formed.

Hydrocyanic acid.—The blood is rendered fluid and bright red ; it kills the protoplasm of the erythrocytes and combines with the hæmoglobin to form cyan-methæmoglobin.

GROUP II

Poisons which have a direct erythrocytolytic action.

The members of this group may be classified as follows :

(1) *Known physical and chemical agents,* e.g. arseniuretted hydrogen, guaiacol.

(2) *Unknown constituents of blood serum.*—Constituents of normal alien serum, eel serum, frog serum.

(3) *Bacterial products.*—Bacterial toxins such as tetanolysin, pyocyanolysin, typholysin, staphylolysin and streptocolysin.

(4) Certain *vegetable poisons* (phytotoxins).—Poisons from mushrooms, phallin, amanitotoxin, helvellic acid, ricin, abrin, crotin, robin ; saponin substances, sapotoxin, cyclamin, solanin.

(5) *Snake venoms* (zootoxins).—From the cobra, water moccasin, copperhead, rattlesnake and others ; scorpion venom, etc.

Arseniuretted hydrogen.—This is a most powerful blood poison, disintegrating the erythrocytes and producing hæmoglobinæmia. The blood in a case I met with resembled that of pernicious anæmia, with marked breaking up of the erythrocytes, polychromasia, basophile granulation, and the presence of megaloblasts and normoblasts (vide Plate II).

Blood serum.—Normal blood serum of some animals causes hæmolysis when mixed with erythrocytes of another animal. It occurs both *in vivo* and *in vitro.* Little is known of their true nature. Eel serum is a powerful hæmolytic, due to a poisonous principle called icthyotoxin. The coagulablity of the blood is greatly reduced.

Bacterial products.—Hæmolytic substances are produced both by pathogenic and non-pathogenic organisms. Hæmolysis occurs as a result of many of the infective diseases, particularly of streptococcal nature. The hæmoglobin is dissolved out and stains the tissues. In chronic cases of infection the secondary anæmia is a result of hæmolysis by the bacterial toxins.

Vegetable poisons.—Some of these are toxalbumins, others glucosides. They act by causing hæmolysis, but some of them possess agglutinating properties which act upon the erythrocytes. Some liberate fibrin ferment and produce thrombi.

Snake venoms (zootoxins).—These produce hæmolysis, and also agglutination of the erythrocytes, but these actions are said, in many cases, to vary in inverse proportion. The greater the hæmolysis the less the agglutination. According to Lamb,[1] if the venom be slowly absorbed the coagulability is decreased and the blood remains fluid, but when rapidly absorbed clotting is increased and thrombosis results. The blood is said to suffer loss of bactericidal power.[2]

GROUP III

Poisons which form methæmoglobin, either while the hæmoglobin is in the erythrocytes or after it has been set free by hæmolysis.

In this group are included potassium chlorate, toluylendiamine, phenylhydroxylamine, nitro-benzine, dinitro-benzine, anilin, and coal tar derivatives such as antifebrin, antipyrin, phenacetin, exalgin, pyrogallol, nitro-glycerine nitrites, amyl nitrite, sulphonal, trional, picric acid, chromic acid, potassium permanganate, iodine.

Potassium chlorate.—The blood is chocolate coloured, the viscosity is increased. Methæmoglobin is present and gives the characteristic spectrum (Fig. 8). Anæmia results from disintegration of erythrocytes, which show marked qualitative changes.

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[1] *Ind. Med. Gazette,* December, 1901.

[2] Welch, *Lancet,* 1894. Ewing, *Med. Record,* 1894.

The coal tar derivatives.—These produce anæmia with disintegration of and qualitative changes in the erythrocytes, in some cases resembling pernicious anæmia. Methæmoglobin is formed.

Nitro-benzine derivatives.—Produce marked hæmolysis, in some cases resembling pernicious anæmia with megaloblasts. The blood is chocolate-coloured and contains methæmoglobin. *Phenylhydroxylamine* is one of the most virulent blood poisons known, with marked hæmolytic action and conversion of hæmoglobin into methæmoglobin. *Anilin* also acts powerfully on the blood, which becomes chocolate-coloured and contains methæmoglobin. It is rapidly absorbed by the skin.

Pyrogallol.—Produces severe anæmia, hæmoglobinæmia, methæmoglobinæmia, and thrombosis.

GROUP IV

Poisons which act by increasing coagulation (or agglutination) in the vessels with the production of thrombi.

In this group are included ricin, abrin, crotin, robin, phosphorus, paraphenyldiamine, snake venoms, silicic acid, corrosive sublimate, certain bacterial products, among which are those of *B. typhosus*, *pyocaneus* and *staphylococcus*, and certain constituents of serum.

Phosphorus.—This produces polycythæmia for a few days, after which the count falls to normal. Agglutination of erythrocytes and thrombosis takes place, and there is usually leucocytosis. Alkalinity is said to be diminished.

Leucocytosis in reference to drugs and poisons has been noted previously (p. 154).

OTHER POISONS WHICH PRODUCE BLOOD CHANGES

Alcohol.—In acute alcoholism leucocytosis is usually present. Hare[1] tested the bacteriolytic power of human serum after administration of alcohol in medicinal doses, against colon bacillus, and found it markedly increased. Mircolo and Gervino[1] found that the blood of alcoholics in whom the effects of the drug had not reached the stage of tissue destruction possesses the power of neutralizing the toxins of the tubercle bacillus. A. C. Abbott[2] from his investigations on the influence of acute alcoholism on the normal vital resistance of rabbits to infection, draws the following conclusions: That the normal vital resistance to streptococcus pyogenes (erysipelas) is markedly diminished; that a similar, though by no means so conspicuous, diminution of resistance to the bacillus coli also occurs. The same is not so pronounced to staphylococcus pyogenes aureus. Alcohol is said to diminish the coagulating power and increase hæmolysis. It produces anæmia. In animals made drunk with alcohol an invasion of the blood by organisms from the intestines may occur.

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[1] *Therap. Gazette*, May, 1903.

Ether.[3]—There is a fall in hæmoglobin during ether anæsthesia, reaching its lowest point twenty-four hours later. One-half of the fall is said to take place during the first hour. Hæmolysis takes place, followed by rapid regeneration of erythrocytes, with an actual increase. Pathological blood is more affected than normal blood. Anæsthesia is dangerous if the hæmoglobin registers less than 50 per cent. A transient leucocytosis is the rule, and myelocytes may appear. Da Costa and Kalteyer[4] record polycythæmia occurring immediately after the termination of the anæsthetic stage.

Chloroform.—Chloroform is said to be retained in the corpuscular portion of the blood to the extent of 20 per cent. The amount of chloroform in the blood during different periods of anæsthesia have been estimated by Byles, Harcourt and Horsley,[5] and in one experiment it was proved that the "fatal dose was not twice the amount required to produce unconsciousness to pain."

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[1] *Gazette deg Osped*, No. 44, 1903.

[2] *Journ. of Experimental Medicine*, vol. i, No. 3, July, 1896.

[3] Anders and Boston, *Therap. Gazette*, November, 1904.

[4] *Annals of Surgery*, September, 1901.

[5] *Brit. Med. Journ.*, July 23, 1904.

Lead.— The action of lead upon the blood has been mentioned in a previous section (p. 107). Anæmia is a constant result and may reach a high grade. Erythrocytes, and particularly hæmoglobin are both diminished, so that the colour index, though inconstant, is low. Qualitative changes in the erythrocytes occur, chief of which is basophile granulation, which comes on rapidly even after a dose of a few grains of acetate of lead and before there is any marked degree of anæmia. Poikilocytes, megalocytes, microcytes, normoblasts, and more rarely megaloblasts, may appear in severe cases. Leucocytosis is usually present, especially in cases of colic.

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